INVESTIGATIONS TO IDENTIFY THE INFLUENCE OF THE INHALATION MANOEUVRE ON THE EX-VIVO DOSE EMISSION AND THE IN-VITRO AERODYNAMIC DOSE EMISSION CHARACTERISTICS OF DRY POWDER INHALERS

Studies to identify the influence of inhalation flow, inhalation volume and the number of inhalations per dose on the ex-vivo dose emission and the in-vitro aerodynamic dose emission characteristics of dry powder inhalers

Sani Ibn Yakubu
MPharm (Clinical); FCPharm

UOB Number: 06013998

Submitted for the Degree of
Doctor of Philosophy

Institute of Pharmaceutical Innovation
School of Pharmacy

2009

Abstract

Investigations to identify the influence of the inhalation manoeuvre on the ex-vivo dose emission and the in-vitro aerodynamic dose emission characteristics of dry powder inhalers

Key words: Ex-vivo, in-vitro, dose emission, aerodynamic characteristics, inhalation, inhalation flow, inhalation volume, dry powder inhalers

Currently available dry powder inhalers (DPIs) for drug delivery to the lungs require turbulent energy to generate and disperse aerosol particles in the respirable range ≤5μm during inhalation. The patient's inspiratory effort together with the resistance inside the device creates this energy. Different inhalers provide varying degrees of resistance to inhalation flow and require different inhalation techniques for the generation and delivery of drug fine particles in respirable size range to the lungs.

The aim of this research programme was to identify the influence of inhalation flow, inhalation volume and the number of inhalations per dose on the ex-vivo dose emission and the in-vitro aerodynamic dose emission characteristics of the salbutamol Accuhaler®, Easyhaler®, and Clickhaler® and the terbutaline Turbuhaler® DPIs.

A high-performance liquid chromatography method for the assay of salbutamol sulphate and terbutaline sulphate in aqueous samples was modified and accordingly validated. In-vitro dose emission of the four different DPIs was measured using the pharmacopoeia method with modifications to simulate varying inhalation flows within patient and between patients. The ranges of the total emitted dose (% nominal dose) at the inhalation flow range of 10 - 60 $Lmin^{-1}$, following one and two inhalations per metered dose for 2L and 4L inhaled volumes were as follows: the Accuhaler (52.64- 85.11; 61.88-85.11 and 59.23-85.11; 62.81-85.11); the Easyhaler (68.35-91.99; 79.94-91.99 and 73.83-92.51; 80.40-92.51); the Clickhaler (46.55-96.49; 51.12-96.49 and 51.18-101.39; 59.71-101.39) as well as the Turbuhaler (46.08-88.13; 51.95-88.13 and 48.05-89.22; 48.64-89.22). The results highlight that the four inhalers have flow-dependent dose emission property to a varying degree using 2L and 4 L inhaled volumes. There was no significant difference in the total emitted dose between a 2L inhaled volume and a 4L inhaled volume at each inhalation flow. Furthermore, the total emitted dose from the Easyhaler®, Clickhaler®, and Turbuhaler® was significantly ($p \leq 0.001$) greater with two inhalations than one inhalation per metered dose across the range of inhalation flow (10 – 60) $Lmin^{-1}$. This effect was only observed at inhalation flow less than 30 $Lmin^{-1}$ with the Accuhaler®. Overall there is a significant difference in the total emitted dose.

The ex-vivo dose emission of the four different DPIs has been determined using the In-Check Dial device to train twelve non-smoking healthy adult volunteers to inhale at slow (30 $Lmin^{-1}$) and fast (60 L min^{-1}) inhalation flows through the device with its dial set corresponding to each inhaler. Subsequently each volunteer inhaled at the trained inhalation flows through each active inhaler. The local ethics committee approval was obtained prior to the study and all volunteers gave signed informed consent. The results obtained demonstrate that the studied inhalers have flow-dependent dose emission, thereby enhancing confidence in the use of the In-Check Dial® to identify a patient's inhalation flows through a variety of DPIs. Also the total emitted dose determined by ex-vivo methodology was significantly ($p \leq 0.05$) greater with two inhalations than one inhalation per metered dose.

The results of the in-vitro aerodynamic dose emission characteristics highlight that the fine particle dose (FPD) from the four studied inhalers is flow dependent. Also the minimum inhalation flow to generate the (FPD) with the appropriate characteristics for lung deposition has been identified to be 20 L min^{-1} for the Accuhaler®, Easyhaler® and Clickhaler®, while that for the Turbuhaler® is about 30 L min^{-1}. Also the inhalation volume above 2L and the number of inhalations for each dose have respectively no significant ($p \leq 0.05$) influence on the FPD emitted from the four studied inhalers. The results support the present instructions to patients using these inhalers to inhale once for each dose as fast as they can.

Acknowledgements

Praise and thanks are Allah`s, the Most Gracious and the Most Merciful.

I would like to express my gratitude to my supervisors Professor Henry Chrystyn and Dr Khalid Hafiz Assi for offering me the opportunity to work with them and their untiring guidance, advice and encouragement throughout my PhD studies.

I would like to thank my volunteers, the secretarial and the school support services staff, especially Angela and her team, in the School of Life Sciences for their help throughout the last three years.

I would like to express my profound gratitude to Admiral Murtala A.H. Nyako for his inestimable support to us. I am grateful to Mahmud H.A. Nyako for all his help to my family.

My appreciations to the University Of Maiduguri, Nigeria for offering me the opportunity to contribute my quota in the academia and for granting me the three-year study leave.

Finally, I am deeply indebted to my wife Fadimatu and children (Ahmad, Dahiru, Yakubu, Adam, Murtala, Yusuf and Aisha) for their untiring encouragement, support, understanding and patience during the period of this study and insha-Allah throughout my whole life.

To my late parents

(May Allah grant them mercy, amen)

List of figures

List of Tables

List of Abbreviations

ACI	Andersen Cascade Impactor
API	Active Pharmaceutical Ingredient
AUC	Area Under The Curve
AVG	Average
BP	British Pharmacopoeia
BTS	British Thoracic Society
CHSR	Committee for Health Services Research
CI	Confidence Intervals
C_{max}	Maximum plasma concentration
COPD	Chronic obstructive pulmonary disease
DPIs	Dry powder inhalers
EP	European Pharmacopoeia
FDA	Food and Drug Administration
FEV_1	Forced expiratory volume in one second
FPD	Fine particle dose
FPF	Fine particle fraction
FPFN	Fine Particle Fraction Nominal dose [%]
FVC	Forced vital capacity
g	Gram
GM-CSF	granulocyte-macrophage colony stimulating factor
GSD	Geometric standard deviation
HFA	Hydrofluorocarbon
HFA-BDP	hydrofluoroalkane-134a beclomethasone
HPLC	High performance liquid chromatography
ICH	International Committee on Harmonisation
kPa	Kilopascal
L	Litre

LD	Laser diffractometry
$Lmin^{-1}$	Litre per minute
LOD	Limit of detection
LOQ	Limit of quantification
mg	Milligram
mg/L	Milligram per litre
mg/ml	Microgram per millilitre
min	Minute
ml	Millilitre
ml/min	Millilitre per minute
mM	Millimolar
MMI	Marple Miller cascade impactor
MMAD	Mass median aerodynamic diameter
MOC	Micro orifice collector
NICE	National Institute for Health and Clinical Excellence
NGI	Next Generation Impactor
PEF	Peak expiratory flow
Eur.Ph	European Pharmacopoeia
PIF	Peak inspiratory flow
pKa	Dissociation constant
pMDIs	Pressurised metered dose inhalers
R^2	Correlation coefficient
RSD	Relative standard deviation
SSGI	Single-Stage Glass Impinger
SIGN	Scottish Intercollegiate Guidelines Network
sec	Second
SPECT	Single photon emission computed tomography
TDPS	Total Dose Per Shot

USP	United States Pharmacopoeia
UV	Ultraviolet
v/v	Volume per volume
v/w	Volume per weight
w/w	Weight per weight
λ	Wavelength
% RSD	Percentage relative standard deviation
°C	Degree Centigrade (Celsius)
µg	Microgram
µl	Microlitre
µm	Micrometre

Chapter 1

1 General Introduction

1.1 Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are common lung diseases that can be treated systemically or by local administration of a bronchodilator and / or a corticosteroid to the lungs via the inhaled route.

The inhaled route of administration is a preferred route for delivering bronchodilators and corticosteroids to patients with asthma and chronic obstructive pulmonary diseases (COPD). In comparison with oral or parental routes of administration, the inhaled route allows delivery of a small but therapeutic dose of drug directly to the airways achieving a high local concentration within the lung, whilst at the same time minimising side effects of the drug. Central to the success of inhaled treatment has been the availability of aerosol delivery systems or inhalers (Chrystyn 2006).

Current available inhalation systems are: nebulisers, pressurised metered dose inhalers (MDIs) and dry powder inhalers (DPIs). Administration of drugs by nebulisation is an effective treatment but only for stationary use and often reserved for patients needing urgent bronchodilator therapy (BTS 1997). MDIs are the oldest and most commonly used inhalation device worldwide because they are, small, portable, and deliver consistent doses up to labelled claim (Boyd 1995). However, side effects due to propellants (cold "Freon" effect that hits the back of throat) may occur (Fink 2000). The main drawback with the MDI is in coordination between the release of the dose and inhalation, necessary for correct dose delivery, leading to reduced effectiveness and poor compliance (Crompton 1982). To overcome the problems with MDIs special add-on devices (spacers) and more recently breath-operated MDIs have been introduced (O'Callaghan, et al, 1997). Also the problems of coordination and formulation, arising from the recently introduced ozone-friendly hydrofluoroalkanes propellants, can be overcome using dry powder inhalers which

are designed such that a patient's inspiratory effort generates particles in the respirable range.

Dry powder inhalers (DPIs) rely on the patient's inhalation flow for drug delivery to the lungs. The patient's inhalation flow interacts with the resistance inside the DPI to generate a turbulent energy which de-aggregates the formulation into an emitted dose containing particles that have the potential for lung deposition (Chrystyn, 2003). The part of the emitted dose from an inhaler device with particles in size range (<5μm) that have the potential to deposit in the lungs is known as the fine particle dose. All DPIs have a different internal resistance (Chrystyn, 2003b) that decreases the inhalation flow generated by a patient. The turbulent energy inside a DPI is represented by a pressure change ($\sqrt{\Delta P}$) that developed across the device during inhalation. This pressure change is directly related to the DPIs` internal resistance to airflow (R) and the inhalation flow (Q) and the relationship is described as: $\sqrt{\Delta P}$ = QR (Clark and Hollingworth 1993). Each type of DPI has its own resistance characteristics which are caused by the internal structure of the device and there is considerable variation in resistance between available DPIs (Steckel and Muller 1997). Since the turbulent energy is a product of the flow and the inhaler's resistance then for a set energy level (inspiratory effort) the flow required through a low resistance DPI will be faster than that of a high resistance DPI. The faster the inhalation flow through a DPI then the greater will be the turbulent energy and therefore the better is the quality of the emitted dose. Hence all DPIs have flow-dependent dose emission with some DPIs more prone to this than others (Chrystyn, 2003c). There is a minimum inhalation flow (hence threshold energy) required at which the de-aggregation is sufficient to provide a dose with the potential to produce particles with the required size. DPIs with higher resistance such as the Easyhaler, Turbuhaler and Clickhaler would require a lower flow whilst for those with a lower resistance (Accuhaler and Novolizer) would require a faster inhalation flow (Assi and Chrystyn 2001).

Different inhalers due to formulations and device designs provide varying degrees of resistance to inhalation flow and require different inhalation technique, i.e. the way in which the patient uses the inhaler. Hence patient information leaflets for DPIs recommended that the inhalation manoeuvre should be as deep and hard as possible.

To ensure effective drug delivery to the lung, the turbulent airstream created in any DPI during inhalation must be sufficient to produce an adequate aerosol cloud with respirable fine particles. This involves a balance between the design of the DPI, the formulation and the patient's generated inhalation flow. A period of breath holding after inhalation improves delivery of inhaled medication because it gives time for the process of sedimentation.

Many patients experience problems using their devices correctly. Poor inhalation technique can markedly reduce the proportion of the drug that reaches the lungs. Studies suggest that 28-68% of patients with asthma have problems using their MDI or DPI sufficiently well to benefit from the dose (Raul 2006). Overall, the issue of correct use of inhalers is of critical importance in maintaining optimal asthma control as patients who misuse their inhalers tend to have less stable control of their asthma than those who use their device correctly (Giraud and Roache 2002).

Thus, the therapeutic efficacy of an inhaled dose depends on the characteristics of the emitted dose, which are a function of a combination of the formulation and the device resistance, intra and inter patient variability and the inhalation technique.

For DPIs the Pharmacopoeias (USP, 2009; EP, 2007; BP, 2008) recommended the use of inhalation volume of 4L at a constant flow corresponding to a pressure drop of 4 kPa across the device to measure both the total emitted dose and the aerodynamic characteristics of the emitted dose, whereas in routine practice patients use varying flows and volumes due to a variety of factors such as lung size (age, gender), degree of airway obstruction that is present and inspiratory musculature (Stocks 1995). Furthermore, when

patients inhale through a DPI, the inhalation volume is less than 4L, for example, it has been reported that the asthmatic and COPD patients have an average inhalation volume of about 2L (Hawskworth et al. 2000).

The Andersen Cascade Impactor (ACI) described in the United State Pharmacopoeia (USP, 2009), European Pharmacopoeia (EP, 2007) and British Pharmacopoeia (2008) has been used in this study to measure the in-vitro characteristics of the emitted dose. Traditionally, the ACI has been designed to operate at flow of 28.3 $Lmin^{-1}$. Use of different flows will alter the cut-off diameter of each stage of the Impactor, whereas patients will inhale at different flows. To overcome this problem, modifications to the stages of the ACI together with the use of a mixing inlet valve have recently been introduced which enabled the measurement of the characteristics of the emitted dose from DPIs at a variety of flows consistent with the patient's routine use.

Previously, Al-Fadhl (2005) used the modified in-vitro method to study the effects of inhalation technique on the emitted dose of tiotropium from a Handihaler (a single dose capsule) dry powder inhaler (DPI). The study highlighted that dose emission was influenced by inhalation flow and volume and that two inhalations were required for each dose. Simulated inhalation flows from a vacuum pump were used in the in vitro study which cannot be extrapolated to the patients' inspiratory flows.

In this research programme, in addition to the in vitro dose emission measurements, a novel but simple ex vivo approach with the aid of the In-Check Dial® to measure the effects of inhalation technique on dose emission from a variety of DPIs has been conceived. The In-Check Dial is a device designed and tested to accurately identify patients` inhalation flows through currently available DPIs (Tarsin, 2000).

Using the in vitro and the ex vivo methods, the flow dependent dose emissions of DPIs have been determined and whether instructions should direct the patient to use one or two inhalations per dose has also been investigated. At present the instructions (manufacturer's

patient information leaflet) for using available multi-dose DPIs state only one inhalation per dose and patients should inhale as fast as they can.

This research programme has focused mainly on four different multi-dose (strips/reservoir type) dry powder inhalers (DPIs) namely-the Ventolin® Accuhaler®, the Asmasal Clickhaler® and the Easyhaler® all containing salbutamol sulphate and the Bricanyl Turbuhaler® containing terbutaline sulphate. Both salbutamol and terbutaline (relievers) are rapid and short acting β2-agonists widely used as the initial drugs of choice for acute bronchospasm in the treatment of asthma and chronic obstructive pulmonary disease (COPD). The British Thoracic Society (BTS) / Scottish Intercollegiate Guidelines Network (SIGN) guidelines recommended short acting β_2 agonists as a first line treatment for the management of asthma, while the National Institute for Health and Clinical Excellence (NICE) guidelines recommended these drugs as a first line option for the management of COPD.

1.2 Hypothesis

According to the Pharmacopoeias, it is recommended that DPIs should be tested using a dose sampling unit and an impactor, using inhalation volume of 4L at a constant flow rate corresponding to a pressure drop of 4 kPa across the device. However, in routine practice patients use varying flows and volumes due to a variety of factors such as lung size (age, gender), degree of airway obstruction that is present and inspiratory musculature (Stocks 1995). It is important to assess the effects of different airflow using different inhalation flows and volumes on the performance of DPIs consistent with routine use by patients.

Also studies have shown that some DPIs operate effectively at peak inhalation flows >30 $Lmin^{-1}$ and that the optimum flows for some DPIs in terms of the total emitted dose and the fine particle dose is >60 $Lmin^{-1}$ (Bisgaard et al. 1998; Nielsen et al. 1998). Other studies have shown that some patients, especially those with COPD (Broeders et al. 2003) and children with severe asthma (Pedersen et al., 1999) were not able to generate the

minimum flows through a DPI that is required to generate an emitted dose with the appropriate characteristics for lung deposition. This means that for these patients, inhaling once from a DPI would result in leaving a portion of the dose in the device and if they inhale twice, they should be able to generate more aerosolised drug particles in the optimal size range to reach the target sites in the lungs irrespective of inhalation flows used. At present there have been no published reports of investigations on the influence of two inhalations per metered dose on the dose emission of multi-dose (strips/reservoir type) DPIs.

The research project was designed to investigate, under the conditions that simulate the patients' routine use of DPIs, the ex vivo flow dependent dose emission properties and the in-vitro emitted dose characterisation from four different multi-dose DPIs (the Ventolin®Accuhaler®, the Asmasal Clickhaler®, the Easyhaler® and Bricanyl Turbuhaler®) and whether instructions should direct the patients to use one or two inhalations per dose. Hence, variable inhalation flows (10 to 60) $Lmin^{-1}$ and inhalation volumes of 2L and 4L respectively, have been used.

1.3 Aims and objectives

The aims of this study were to:

- Assess by in-vitro methods the effects of different inhalation flows and inhalation volume on the emitted dose and the aerodynamic characteristics of the emitted dose from the Accuhaler®, the Easyhaler®, the Clickhaler® and the Turbuhaler®.
- Evaluate by in-vitro methods whether there is difference in the emitted dose and the aerodynamic characteristics of the emitted dose from the above mentioned inhalers, when inhaling once or twice per dose.
- Measure using ex-vivo methodology, the effects of inhalation flow on the total dose emission and whether there is a difference in the emitted dose from the above mentioned inhalers, when inhaling once or twice per dose.

The objectives were to:

- Validate a high performance liquid chromatography (HPLC) method for the assay of salbutamol sulphate in aqueous samples.
- Validate a high performance liquid chromatography (HPLC) method for the assay of terbutaline sulphate in aqueous samples.
- Determine the in-vitro emitted dose of salbutamol sulphate from (the Ventolin®Accuhaler®, the Asmasal® Clickhaler®, and the Easyhaler®) as well as terbutaline sulphate from Bricanyl® Turbuhaler® following one and two inhalations per dose at varying inhalation flows (10 to 60) $Lmin^{-1}$ for inhaled volume of 2 and 4L respectively.
- Determine the in-vitro characteristics of the emitted dose of salbutamol sulphate from (Ventolin®Accuhaler®, Asmasal® Clickhaler®, and Easyhaler®) as well as terbutaline sulphate from Bricanyl® Turbuhaler® following one and two inhalations per dose at varying inhalation flows (10 to 60) $Lmin^{-1}$ for inhaled volume of 2 and 4L respectively.
- Determine with the aid of the In-Check Dial® the ex-vivo emitted dose of salbutamol sulphate from (Ventolin®Accuhaler®, Asmasal® Clickhaler®, as well as Easyhaler®) and terbutaline sulphate from Bricanyl® Turbuhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows respectively, performed by healthy volunteers.

1.4 Thesis structure

The thesis consists of seven chapters:

Chapter 1 is a general introduction highlighting the principles governing the operation of aerosol delivery devices, especially dry powder inhalers and the factors that affect the delivery and deposition of aerosolised drug particles to the target site in the lungs. Previous studies on the effects of inhalation flows, inhalation volume and two inhalations on dose

emission of (a single dose capsule type) have been highlighted. This provides the rational for this research work. Also the aims and objectives of the research have been included.

Chapter 2 provides an overview of the issues related to this research work which includes:

- Respiratory system-including brief physiology and anatomy.
- Asthma and chronic obstructive airways pulmonary disease (COPD) and their management. This details the use of various type of bronchodilators (ß-agonists and anticholinergics) and corticoid steroids used in the prevention and management of these diseases.
- Inhalation therapies: introduction to the importance of inhalation therapies, drug delivery system, mechanism of particle deposition, factors influencing deposition of particles, use of the In-Check Dial to identify inhalation rates, and the methods to determine bioequivalence.

Chapter 3 details the HPLC method for the assay of salbutamol sulphate and terbutaline sulphate in aqueous samples.

Chapter 4 focuses on the in-vitro dose emission of salbutamol sulphate from the Accuhaler®, the Clickhaler® and the Easyhaler® respectively and terbutaline sulphate from the Turbuhaler®.

Chapter 5 describes the determination of the in-vitro aerodynamic particle size distribution of salbutamol sulphate from the Accuhaler®, the Clickhaler® and the Easyhaler® and terbutaline sulphate from the Turbuhaler® at varying inhalation flows (10 to 60 $Lmin^{-1}$), following one and two inhalations for each dose using inhalation volumes of 2L and 4L respectively.

Chapter 6 describes the measurement of the ex-vivo dose emission of the four different DPIs with the aid of the In-Check Dial® using healthy volunteers at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows following one and two inhalations.

Chapter 7 provides a general discussion and conclusion.

Chapter 2

2 Literature review

2.1 Respiratory system

The efficacy of aerosol therapy depends on the ability of an inhaler to deliver sufficient drug of suitable-particles to appropriate sites in the lungs with minimal side effects (Pauwels R et al. 1997). This is, in turn, depends on aspects of airway anatomy and physiology, which will alter with age and disease status (Martin et al. 1988; Stocks 1995; Wohl 1998) and therefore need to be understood in considering both delivery and deposition.

The human respiratory tract is a branching system of air channels with more than 23 bifurcations from the mouth to the alveoli that looks like an inverted tree with a single trunk (Figure 2.1). The most widely used morphologic model for describing the structures (Table 2.1) within the lung was initially given by (Weibel 1963; Hickey 1992). The first region is the upper respiratory tract, which includes the nose, mouth and pharynx. The main function of this region is heating and moistening of air as well as acting as a filter. Normal atmospheric air contains around 40 - 60% moisture and usually has a temperature of 20 °C. In the mouth, nose and throat the air is heated to 37 °C and moistened to 99% relative humidity. The pharynx is the common opening of both the digestive system and the respiratory system. It receives air from the nasal cavity and air, food and water from the mouth. Inferiorly the pharynx connects the respiratory system at the larynx and the digestive system at the oesophagus. The larynx consists of an outer casing of nine cartilages that are connected to each other by muscles and two pairs of ligaments. The epiglottis prevents food and liquid from entering the larynx and air from leaving the lung.

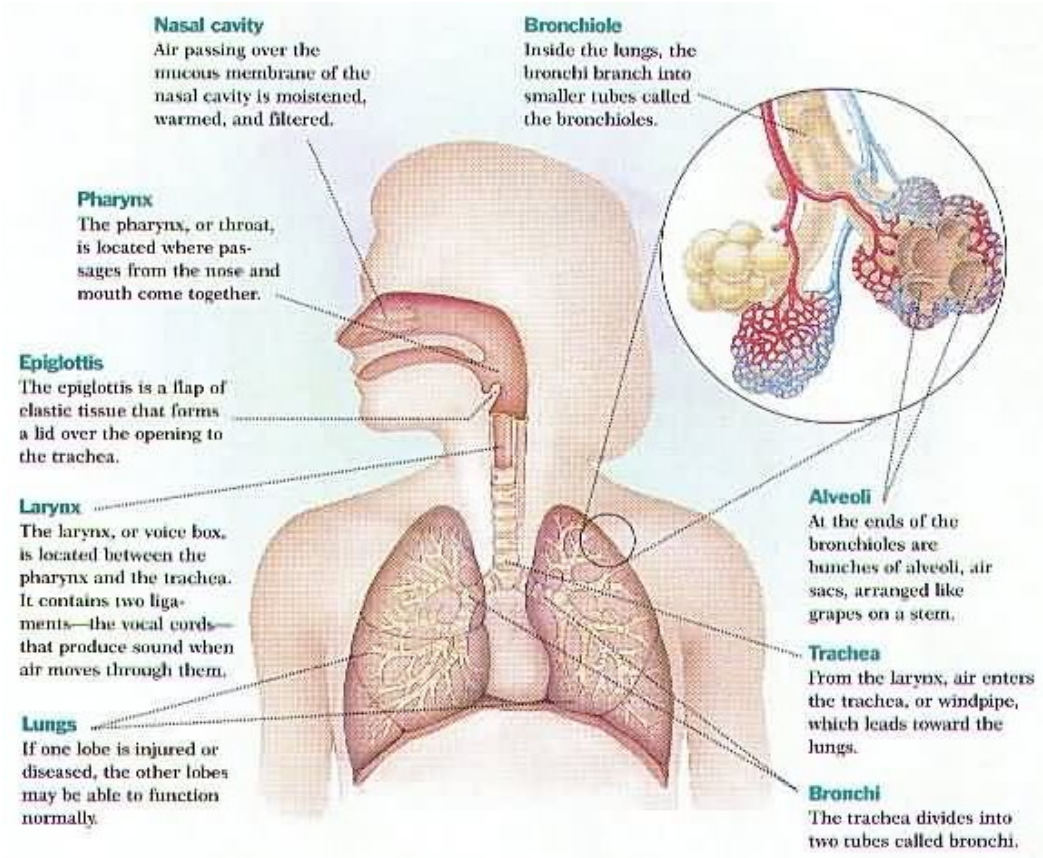


Figure 2.1 Physiology of lungs (adapted from Massachusetts General Hospital Cancer Resource Room, Boston, USA).

The second region is the conduction zone. This region consists of the first 16 generations of branching. The airways of the conducting zone are described as rigid tubes that initially consist primarily of cartilage in the walls. These airways symmetrically divide or bifurcate beginning with the trachea and ending with the terminal bronchioles (Figure 2.1). The third region is the transitional zone. This region consists of generations 17 through to 19 of the branching (Figure 2.1). The respiratory bronchioles each consist of a few alveoli in which limited gas exchange occurs. The fourth region is the respiratory zone. This region consists of generations 20 to 23 of the branching, ending in the alveoli. In the highly vascularised respiratory zone gas exchange occurs by adding oxygen to, and removing carbon dioxide from the blood passing the pulmonary capillary bed. With increasing generation number, the number of branches rapidly increases, while the distance between the branches and the airway diameter decrease. The summed cross sectional area from the mouth to the alveolar

sacs rapidly increases and results in a trumpet shaped lung model, with a total absorptive surface area of up to 100 m^2 (Hickey 1996)

Table 2.1 Morphorlogic model for describing the respiratory tract

	Generation		Diameter (cm)	Length (cm)	Number	Total cross sectional area (cm^2)	Powder deposition by particle diameter
Conducting Zone	*Trachea*	0	1.80	12.0	1	2.54	7-10 µm
	Bronchi ↓	1	1.22	4.8	2	2.33	2-10 µm
		2	0.83	1.9	4	2.13	
		3	0.56	0.8	8	2.00	
	Bronchioles ↓	4	0.45	1.3	16	2.48	
	Terminal bronchioles	5 ↓ 16	0.35 ↓ 0.06	1.07 ↓ 0.10	32 ↓ $6x10^4$	3.11 ↓ 180	
Transitional Respiratory Zones	*Respiratory bronchioles*	17	↓	↓	↓	↓	0.5 – 2 µm and < 0.25 µm
		18	0.05	0.10	$5x10^5$	10^3	
		19					
	Alveolar Ducts	20	↓	↓	↓	↓	
		21					
		22					
	Alveolar sacs	23	0.04	0.05	$8x10^6$	10^4	

2.1.1 Pulmonary volumes, capacity and indices*:*

The inhaled air volume depends on the extent (Kresch 1996) of chest enlargement. During normal breathing, the inhaled and exhaled volumes (tidal volume) are only a part of the

total lung volume. The different parameters describing pulmonary ventilation are shown in Figure 2.2. Definitions of the different parameters are given in Table 2.2.

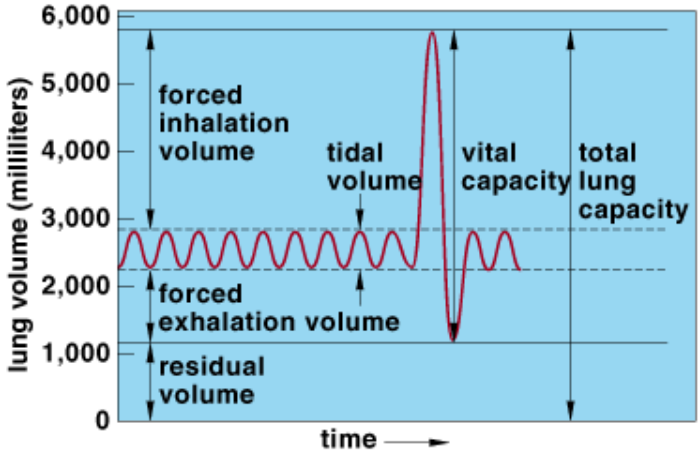


Figure 2.2 Spirometric tracing for lung volumes and capacities (Hickey 1996).

Determination of lung volumes and capacities can provide important information on the pathophysiological status of the lung. The amount of air moving in and out of the lungs (characterised by VT, IRV, ERV, VC and IC) can be measured through spirometry. Definitions for the abbreviation used and described below in Table 2.2. Estimates for the volume of air remaining in the lungs after expiration (RV and FRC) are made by gas dilution methods and by body plethysomography. The respiratory system of a normal adult processes 10-20 m^3 of air per day. Furthermore performing a vital capacity manoeuvre with as much force as possible provides useful data. These spirometric measurements are the Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 second (FEV_1) and the Peak Expiratory Flow Rate (PEFR). The gas-exchange area of the lungs is about 120 - 160 m^2 and is perfused with over 2000 km of capillaries (Hickey 1996). At rest, about 500 ml of tidal air is inhaled and exhaled with each breath (Hinds 1982). During heavy work, tidal volume may be three times as much. A resting adult breathes about 12 times per minute and this rate will triple during heavy work.

Table 2.2 Definitions of lung volumes and capacities describing pulmonary ventilation

Parameter	**Definition**
Tidal volume (VT)	The volume of air inspired or expired during a normal breath
Inspiratory reserve volume (IRV)	The maximal volume of air that can be inspired after a normal tidal inspiration
Expiratory reserve volume (ERV)	The maximal volume of air that can be expired after a normal tidal expiration
Residual volume (RV)	The volume of air remaining in the lungs after a maximal expiratory effort
Inspiratory capacity (IC)	The maximal volume of air that can be inspired after a normal tidal expiration (IC = VT + IRV)
Functional residual capacity (FRC)	The volume of air remaining in the lungs after a normal tidal expiration (FRC = ERV + RV)
Vital capacity (VC)	The maximal volume of air that can be expired from the lungs after a maximal inspiration (VC = IRV + VT + ERV)
Total lung capacity (TLC)	The volume of air in the lungs after a maximal inspiratory effort (TLC = IRV + VT + ERV + RV) ml

2.1.2 Pulmonary mechanics

The ventilatory apparatus consists of the lungs and surrounding chest wall. The chest wall includes not only the rib cages but also the diaphragm and abdominal wall (Figure 2.3).

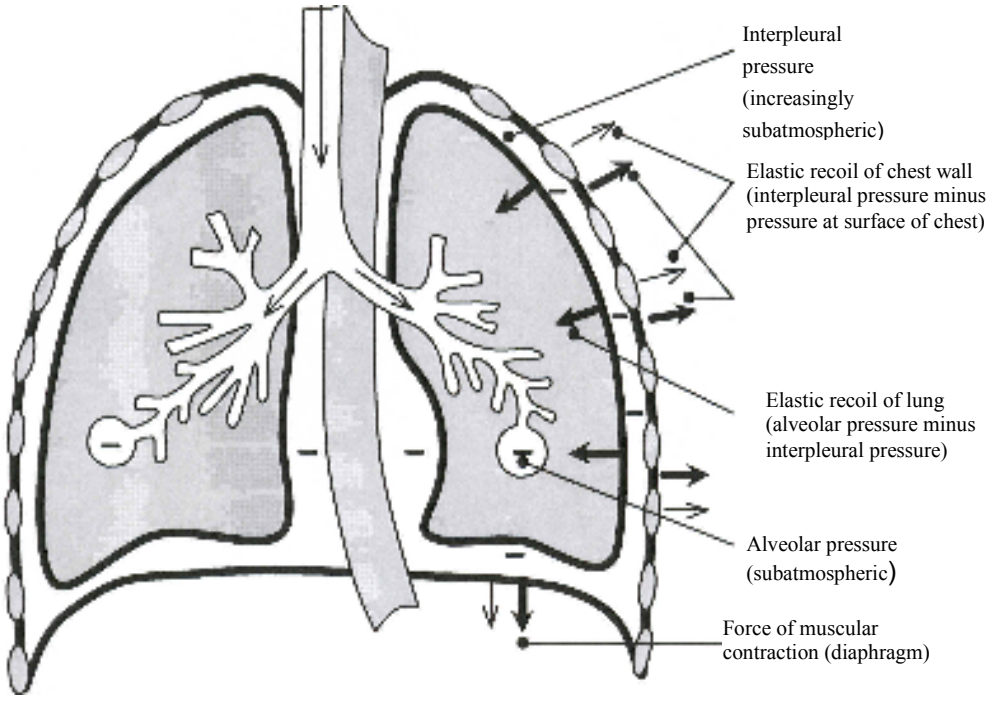


Figure 2.3 Forces and pressures during inspiration (Illustration from CIB collection of Medical Illustrated by F.H Netter, M.D. 1979)

Movement of air into and out of the lungs is driven by pressure differentials or gradients across the lungs. When inspiratory muscles (diaphragm and intercostal muscles) contract to expand the thoracic cavity, a force is applied to the lung surface, which causes expansion of the lungs. Lung expansion occurs because the lungs are compliant and distensible. By expanding, a negative pressure is created within the lungs, specifically in the airways and alveoli. This results in airflow in the direction from high to low pressure, which is in the direction of the alveoli. Changes in lung pressures relative to atmospheric pressure can be summarised as follows. At the start of inspiration, the alveolar pressure equals atmospheric pressure. There is no pressure difference and thus, no driving force for airflow. In this situation, the relative alveolar pressure is referred to as zero (Figure 2.4). Interpleural

pressure is about –500 Pa because elastic recoil of the lungs counteracts the forces of the chest wall to recoil outwards. Thus, a negative pressure is generated in the interpleural space between the lungs and the chest wall. Upon inspiration, a greater negative intrapleural pressure is generated as the chest wall moves outward against the elastic recoil of the lungs, reaching a maximal value of about –700 to –800 Pa under normal conditions. The expansion of the lungs by the greater negative intrapleural pressure causes alveolar pressure to decrease (become negative relative to atmospheric pressure) until it reaches a maximum value of about –100 Pa under normal conditions, providing the pressure gradient for air to flow into the airways and alveoli (depicted as negative flow in Figure 2.4).

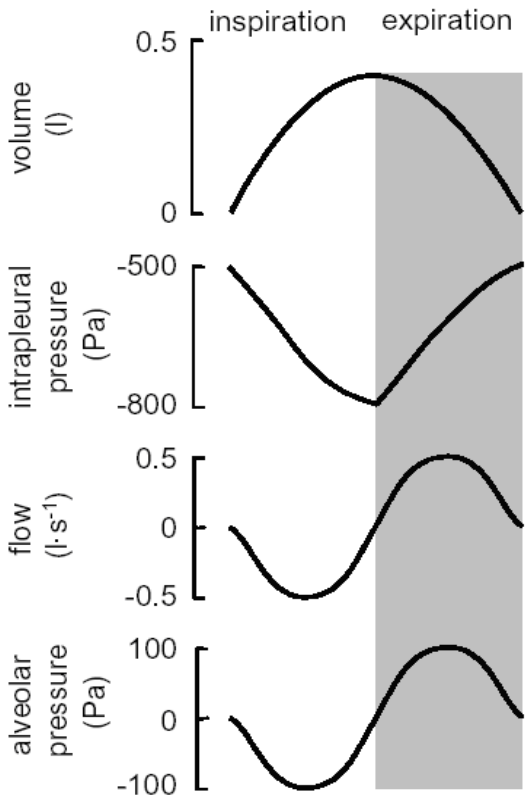


Figure 2.4 Ventilatory parameters during tidal breathing (single breath) (Hickey 1996)**.**

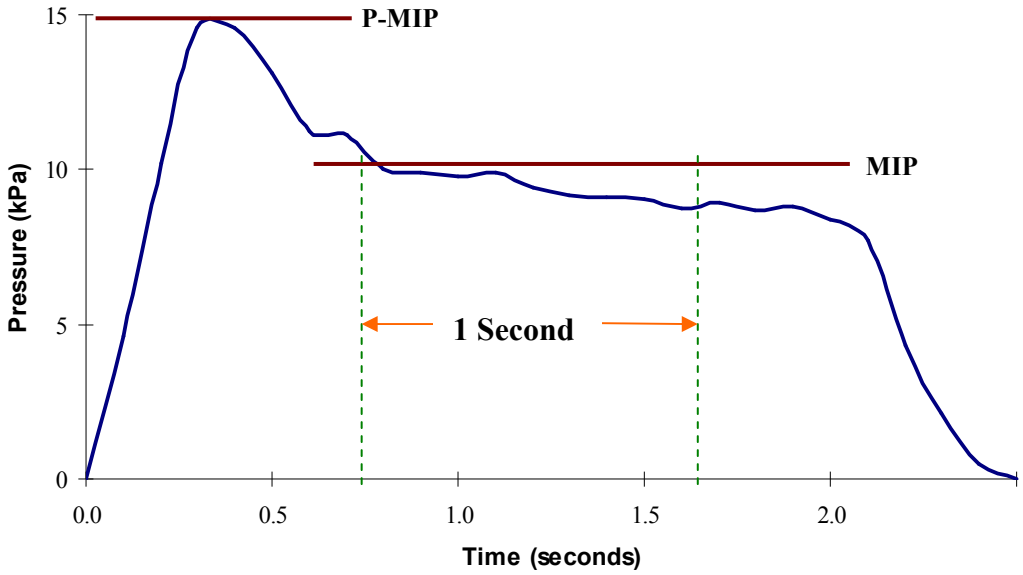
The rate of airflow does not depend on the pressure gradient alone but also on the internal resistance to airflow of the airway system, which is mainly a function of the airway diameters and the existence of obstruction. The obstructions reduce the airway diameters locally, thereby increasing the resistance to airflow.

Patients suffering from obstructive diseases have to generate higher pressure differences to create the same airflow rate, compared to patients without lung obstructions. Consequently, alveolar pressures have to be much lower. During inhalation, the airflow gradually decreases as the alveoli are filled with air and the relative alveolar pressure returns to zero. The difference between intrapleural pressure and alveolar pressure is the transpulmonary pressure, which provides a measure of the elastic lung recoil at each point of lung expansion. When inspiration is complete and the lungs are inflated, respiratory muscles relax and the elastic recoil properties of the lung cause it to return to its original state prior to inflation, thereby expelling the inspired air. Intrapleural pressure returns to –500 Pa and alveolar pressure increases to about +100 Pa, thereby creating the pressure gradient to allow air to flow out of the lungs to the external environment (depicted as positive flow in Figure 2.4). Throughout this cycle of normal inspiration and expiration, airways remain open in order to allow air to flow in and out of the lungs with relative ease.

2.1.3 Inspiratory muscle strength

The maximal inspiratory mouth pressure is a measure for the inspiratory muscle strength. In this measurement, maximal inspiratory manoeuvres from residual volume (RV) are performed against a closed shutter. An oval flanged mouthpiece with a small leak is used to prevent the use of the buccinator muscles (Black and Hyatt 1969; Koulouris et al. 1988; Mayos et al. 1991). Conventionally, inspiratory muscle strength has been assessed by maximal inspiratory mouth pressure sustained for one second (PI_{max} or MIP) during a maximal static manoeuvre against a closed shutter (Black and Hyatt 1969; Leech et al. 1983; Wilson et al. 1984; Newell et al. 1989; Bruschi et al. 1992; Nava et al. 1993).

However, PI_{max} is poorly reproducible (Fiz et al. 1989; Wen et al. 1997). The peak maximal inspiratory pressure (P-MIP) during a maximal static manoeuvre is a more valid assessment of inspiratory muscle strength. This measurement is considered to be less influenced by the learning effect and has a high reproducibility (Larson et al. 1993; Wijkstra et al. 1995). Figure 2.5 shows a typical pressure recording during fast maximal inhalation obtained from a maximal inspiratory pressure measurement. The peak value is referred to as the peak maximal inspiratory pressure (P-MIP) or P-PI_{max} (Larson et al. 1993; Wijkstra et al. 1995). The plateau value, which has to be maintained for at least one second, is referred to as the maximal inspiratory pressure (MIP). As shown in Figure 2.5 the flow profile in the mouth follows the alveolar pressure profile during ventilation without time delay. The commonly used inhalation-instruction for dry powder inhalers is to inhale forcefully and deeply. Because the airflow through breath controlled dry powder inhalers depends on the patient-generated inspiratory pressure, the P-MIP might be a useful measure for the peak inspiratory flow through a dry powder inhaler.



.

Figure 2.5 Diagram with definitions of peak maximal inspiratory pressure (P-MIP) and maximal inspiratory pressure (MIP).

In some studies an indication is given that the inspiratory muscle strength might be a determinant for the peak inspiratory flow through breath-controlled DPI's, or a resistance to airflow (Rainer et al. 1992; Clark and Bailey 1996; Sarinas et al. 1998). Moreover, the respiratory muscles are striated skeletal muscles, which can be trained like other striated muscles in order to increase their strength and endurance. This is demonstrated in healthy volunteers (Leith and Bradley 1976; O'Kroy and Coast 1993) as well as in patients (Dekhuizen et al. 1991; Reid and Dechman 1995; Villafranca et al. 1998) training with high force contractions increases maximal force, whereas training with high velocity, low force contractions, increases maximal shortening velocity (Reid and Samrai 1995; Tzelepis et al. 1999).

2.2 Asthma

Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils, and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or early morning. These episodes are associated with widespread and variable degrees of airflow obstruction that is often reversible either spontaneously or with treatment. The inflammation causes an associated increase in the existing bronchial hyper responsiveness (BHR) to a variety of stimuli (NHLBI 1997).

Asthma has become one of the commonest chronic diseases in the industrialised countries. Its prevalence has increased over the past 20 years with a dramatic increase in hospital admission rates and general practitioner consultations, for both adults and children (Hill and Thomason 1998). Children have the highest prevalence (Akinbami and Schoendorf 2002). A report indicates that there are 5.2 million people in the UK with asthma, with 1.1 million of these being children, making it the most common long-term medical condition with 1 in 10 children being affected. 70,000 people are admitted to hospital in England

with asthma attacks and there is one death every 7 hours from asthma. Asthma is estimated to cost the UK over £2.3 billion per year, which includes both the cost to the NHS and lost productivity due to absence from work (Asthma UK, 2004).

2.2.1 Causes of asthma

Although genetic disposition to atopy (tendency to form immunoglubin IgE antibodies against common materials present in environments) is a significant risk factor for developing asthma, the underlying causes of the disease are not known. What is known is that an individual's environment appears to be important in determining whether the individual becomes asthmatic or not. The environmental risk factors for the development of asthma include exposure to maternal smoking during pregnancy and infancy, exposure to concentration of allergens (air bone pollen, house-dust-mites, animal dander), viral infection during infancy, air pollution, food preservatives and drugs (von Matius 2000) .

2.2.2 Pathophysiology

Airways inflammation, as seen in asthma, is a complex and dynamic process with acute and chronic events occurring simultaneously. Inhaled allergen challenge models, as demonstrated in extrinsic asthma, contribute to the understanding of acute inflammation in asthma (Kay 2001).

Patients with extrinsic asthma tend to be atopic, that is, they have high level of circulating immunoglobulin IgE antibodies against allergens, produced by T-lymphocytes due to previous/chronic priming of the immune system (Davies 1998). These immunoglobulin bind to the mast cells present in the epithelial layer of the airways. Inhalation of allergens further stimulates lymphocytes to produce more IgE. Also the allergen binds to the mast cell-bound IgE and induces de-granulation of the cell. De-granulation causes the release of histamine and other mediators such as prostaglandins, neutrophils, chemotactic factors and leukotriene which cause a sudden bronchoconstriction of the airways (immediate asthma

response). The other consequence of the release of these mediators is an action on the microvasculature causing oedema and airways narrowing (Kays, 2001). Activation of lymphocytes also causes the release of cytokines such as interleukin-5 and granulocyte-macrophage colony-stimulating factors (GM-CSF) that are involved in inflammatory cell recruitment and activation. Some cytokines enhance production of IgE while others such as interleukin-5 are involved with recruitment of eosinophil and macrophages from the circulation into the airways (Busse and Lemanske 2001). Eosinophil secrets toxic substances such as eicosanoids, super oxides and eosinophil cationic protein (ECP) that destroy allergens, while causing damage to epithelial and endothelial tissues of the airways. The release of mediators from the eosinophil stimulates mucus production from the goblet cells and from the deeper ducts in the airways. All these later processes are characterised by the late asthmatic responses (LAR). Continued stimulation of the inflammatory process by allergens either as symptoms or acute exacerbation leads to an initiation of the chronic inflammatory processes where damage to the airway tissues exceeds repair and airways re-modelling occurs. Also exposure of nerve endings which leads to twitching airways, causes further bronchoconstriction (hyperresponsiveness). Extrinsic asthma also known as allergic, episodic or early-onset asthma is commonly seen in children.

Intrinsic asthma, which involves late-onset, is characterised by unknown or poorly defined agents, circumstances, or conditions responsible for attacks. Patients are not atopic and the condition is usually found in adulthood (Green and Harris 2000). Aspirin and non-steroids anti- inflammatory drugs (NSAID) may precipitate potentially fatal asthmatic attacks in 8-20% of patients with asthma (Sturevany 1999). This syndrome known as aspirin-induced asthma can include bronchospasm, rhinorhea, dyspnoea, cough and angiodema. It usually develops over a period of 20 minutes to three hours after ingestion of the causative agent.

Exercise- induced bronchospasm following either drying or cooling of the airways occurs in most patients with asthma (Busse and Lemanske 2001).

2.2.3 Diagnosis

A detailed medical history and spirometry, are usual to establish the presence of episodic symptoms of airway obstruction (Davies 1998). Methods used to obtain objective measurement of lung function include peak expiratory flow rate (PEFR) and forced expiratory volume during the first second of a forced expiratory vital capacity test (FEV_1). Ideally, people who have not smoked or do not have asthma should be able to blow out at least 70-75% or more of their total lung capacity within the first second of a forced exhalation (FEV_1). Reduction in the FEV_1 (less than 80% of the total forced volume) indicates an obstructive lung disease (Hughes and Pride 2000).

Traditionally, the diagnosis of asthma is based on the demonstration of greater than 15% improvement in the FEV_1 or peak expiratory flow rate (PEFR) following the inhalation of a bronchodilator. Measurements of PEFR on waking, in the middle of the day, and before bed are also useful in demonstrating the variable airflow obstruction that characterise asthma. A drop of 20% in the FEV_1 which occurs after the inhalation of a small amount of methacholine or histamine indicates the presence of bronchial hyper-reactivity.

2.2.4 Management of asthma

The British Thoracic Society (BTS 2007) has recommended a 5-step approach for the management of asthma as shown in the Figure 2.6. The stepwise approach to treatment is based on the use of inhaled short-acting β2-agonists (SABA) such as salbutamol and terbutaline (relievers) as the first line of treatment followed by inhaled corticosteroids (preventers). The treatment gradually increases to the addition of a long acting β2-agonist (LABA) until the final step where oral corticosteroids are used for more severe cases and during exacerbation as a short course.

Step 1 Relievers such as salbutamol, terbutaline are short acting $beta_2$ agonists (SABA), quick-onset drugs which produce rapid relief from the symptoms of asthma. These are

inhaled as required. Using two or more canisters of beta$_2$ agonists per month or >10-12 puffs per day is a marker of poorly controlled asthma (SIGN 2002). If the patient is using a SABA on a regular basis anti-inflammatory therapy is recommended.

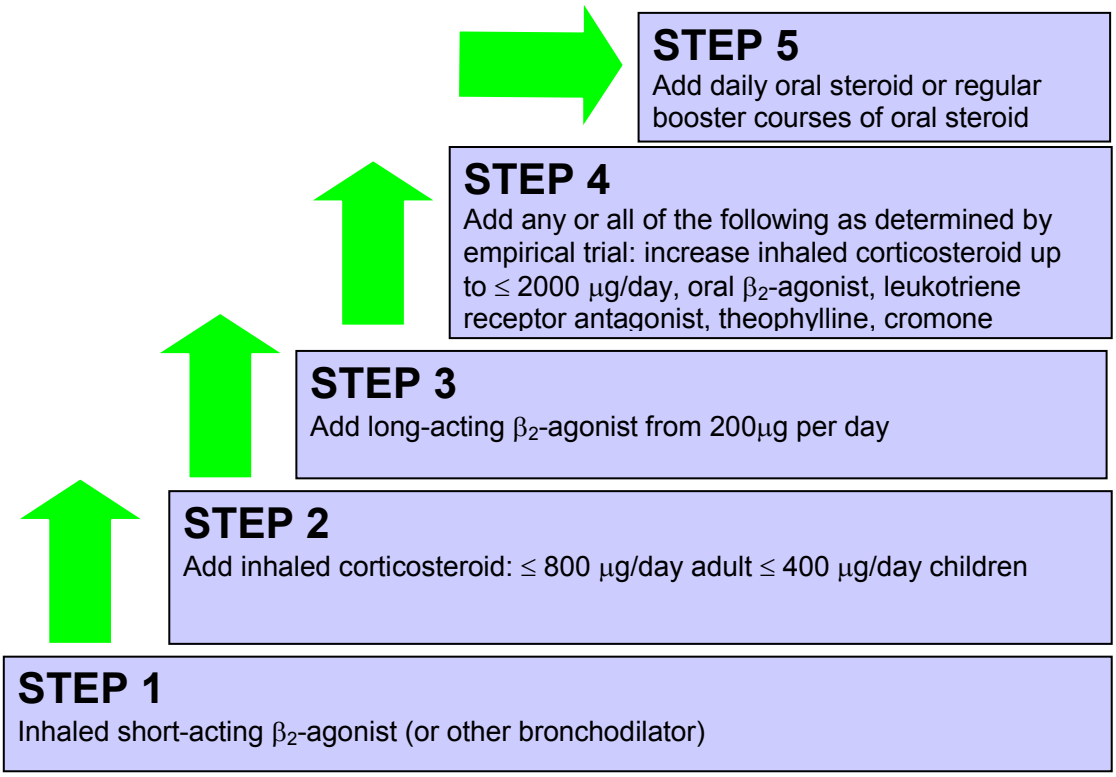


Figure 2.6 Proposed BTS / SIGN stepwise approach for the management of asthma (www.brit-thoracic.org.uk).

Step 2 has been judged on the ability to improve symptoms, improve lung function, and prevent exacerbations, with an acceptable safety profile. Inhaled steroids are the most effective preventer drugs for asthmatic patients for achieving overall treatment goals (Adams et al. 2001; SIGN 2002). Two recent studies have shown benefit from regular use of inhaled steroids in patients with mild asthma (O'Byrne et al. 2001; Pauwels 2003). Inhaled steroids should be considered for patients with any of the following:

- Exacerbations of asthma in the last two years
- Using inhaled beta$_2$ agonists three times a week or more
- Symptomatic three times a week or more, or waking one night a week.

Literatures also suggest the use of twice daily dosing is more effective than single dose (CHSR 1999; SIGN 2002).

Step 3 focuses on add on therapy along with steroids. The use of high doses of steroids can cause side effects in patients. The BTS (2007) guidelines recommend a trial with add on medications before stepping up the dose of steroids.

The first recommended choice is the use of long acting $beta_2$ agonists (LABA) like formoterol or salmeterol to improve lung function and symptoms, and decrease exacerbations (Becker and Simons 1989; Kips and Pauwels 2001; SIGN 2002).

Step 4 advises an increase in the dose of steroids if there is poor control when prescribed a moderate dose of inhaled steroid from MDI with spacer. If there is a response to LABA, but control remains poor, LABA should be continued and the dose of the inhaled steroid should be increased. If the addition of add on still remains inadequate, the use of leukotriene receptor antagonists, theophyllines, slow release $beta_2$ agonist tablets are recommended in step 4 (Ducharme and Hicks 2002; SIGN 2002). LABA should always be used with an inhaled steroid and not alone in the management of asthma.

There is also a recommendation to add leukotriene receptor antagonists and theophyllines, however the side effects are considered to be high. The maximum inhaled steroid dose that is recommended is equivalent to beclomethasone 2000 µg / day.

Step 5 recommends the use of oral steroids using the lowest dose that provides adequate control. A high dose of inhaled steroid at 2000 mcg/day is advised.

The treatment of asthma in children is similar to the treatment in adults with some modifications. It is more essential that airway inflammation be minimised or prevented in younger patients, because years of persistent inflammation can increase the potential for the chronic effect of inflammation, which causes airway re-modelling and the development of irreversible chronic obstructive airway disease at an early stage (Gershwin and Albertson, 2001).

Central to the management of asthma is careful and continual monitoring. Patients should receive regular clinic review, be encouraged to participate in the monitoring of their condition by means of PEFR recordings and be able to tailor their therapy to their level of symptoms. As an important prophylactic measure, patients with asthma should avoid triggers such as aero-allergen sources (e.g. pollen, house dust mites). It is recommended in the guidelines that compliance and inhalation technique are checked before making changes to a patient's therapeutic management.

2.3 Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease (COPD) is a disease state characterised by airflow limitation that is not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases. The disease is predominantly caused by smoking [(National Institute for Health and Clinical Excellence (NICE) 2004; Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2007; NICE 2004)]. The most common conditions comprising COPD are chronic bronchitis and emphysema. Chronic bronchitis is defined as a condition with presence of cough and sputum production for at least 3 months in each of 2 consecutive years, is not necessarily associated with airflow limitation (NICE, 2004b).

2.3.1 Causes

Irritants like cigarette smoke, air pollution, or infection can produce a chronic inflammation of the bronchi (Jensen et al. 2000). If the irritant persist then the diameter of the bronchi decreases and ventilation is impaired causing bronchitis. There is an increase in mucus production in some patients that leads to chronic bronchitis. Also emphysema results in the destruction of the alveolar walls. Loss of the alveolar walls decreases the respiratory membrane surface area, decreasing the gas exchange and loss of elastic fibers that decrease the ability of the lung to expel air out. Figure 2.7 reproduces classic data from

a study by Fletcher et al (1977) showing the different rates of decline in the FEV_1 with age for non-smokers and smokers who either do or do not develop COPD. The horizontal lines have been added to show the boundaries of COPD severity recommended by the global initiative on obstructive lung disease (GOLD) (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2007). These investigators also showed that in a susceptible minority of tobacco smokers (estimated at 15–20% of the total), lung function declines rapidly to levels consistent with moderate (GOLD 2), severe (GOLD 3), and very severe (GOLD 4) COPD. Their data also showed that stopping smoking had a beneficial effect on stopping the fast rate of decline in the FEV_1 at any age.

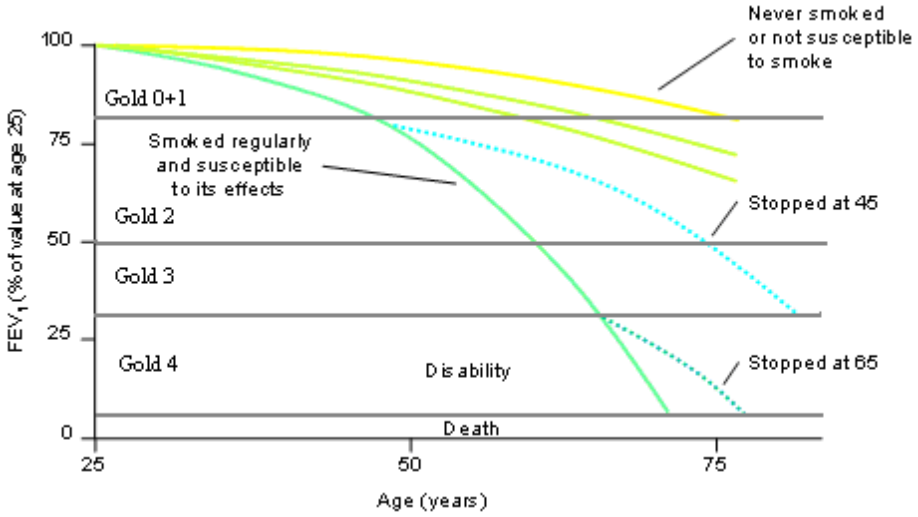


Figure 2.7 Natural history of chronic obstructive pulmonary disease at varying age population [Reproduced from (Fletcher and Peto 1977)].

Although this shows rate of loss of FEV_1 for one particular susceptible smoker, other susceptible smokers will have different rates of loss, thus reaching disability at different ages (Fletcher and Peto 1977) as lung inflammation is present in everyone with a tobacco smoking habit (Hogg 2004). The reason why only a minority of smokers experience an excessive decline in FEV_1 is unknown, but preliminary evidence suggests that the lung inflammatory response is amplified in the susceptible group (Retamales et al. 2001). Figure 2.7 highlights why smoking cessation is the first intervention for COPD patients as recommended by the NICE guidelines, whereas long-term oxygen therapy is able only to

prolong survival in severe COPD patients (Gorecka et al. 1997). Associated risk factors for COPD include:

- Tobacco exposure.
- Alpha-1 antitrypsin deficiency.
- Occupational exposure eg cadium, silica or dusty environments.
- Low social class.
- Diet deficient in vitamin C.
- Pre-existing bronchial hyper-responsiveness.
- Low birth weight.
- Childhood respiratory infections.

However, there are some smokers that are not at risk, for reasons that are not fully understood. This may be related to an individual's genetic profile that gives rise to α_1 antitrypsin deficiency with resultant low levels of protease inhibitors in those smokers who develop COPD (Figure 2.9). Non-smokers that develop COPD usually have a deficiency of alpha-antitrypsin.

COPD affects 600 million people worldwide and is, at present, the sixth leading commonest cause of death worldwide and the fourth leading cause of death in developed countries. In the UK nearly 900,000 people are diagnosed as having COPD, and it was the fifth common cause of mortality after coronary heart disease, pneumonia, stroke and caner (BTS, 2001). A progressive increase in COPD has occurred between 1990 and 1997 with a sharp increase in number of hospital admissions due to COPD from 1995 to 1999 as shown in Figure 2.8. The trend in the number of hospital admissions due to COPD might not have been overcome to date. It is the only common chronic disease that has an annual mortality rate that is still increasing. In 1999 there were approximately 30,000 deaths due to COPD in the UK. This represented 5.1% of all deaths, with approximately 20 times as many deaths compared to asthma.

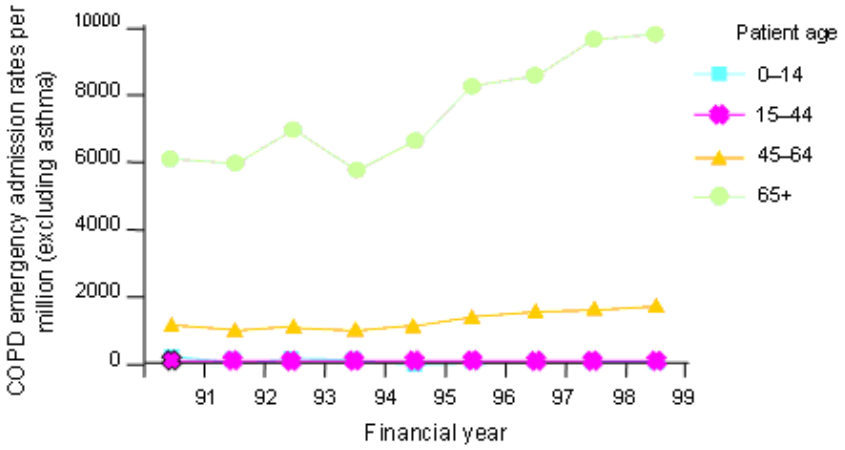


Figure 2.8 Hospital admissions due to chronic obstructive pulmonary disease 1990-99 (Adapted from Lung and Asthma Information Agency, Fact sheet 2001/4).

2.3.2 Pathophysiology

The major players in the inflammatory changes in COPD are: neutrophils, macrophages and CD8+ lymphocytes. The actions of these cells and mediators are complimentary, leading to widespread destructive changes (NHLBI, 2001). The stimulus for the activation of inflammatory cells and mediators is an exposure to noxious particles and gas through inhalation. Other processes involved in the pathogenesis of COPD include oxidative stress and an imbalance between aggressive and protective defence system in the lung (protease and anti proteases). The increased oxidants generated by cigarette smoke react with and damage various proteins and lipids, leading to cell and tissue damage. Oxidants also exacerbate the protease-anti proteases imbalance by inhibiting antiproteases, that is, α_1 antitrypsin (AAT) (Figure 2.9).

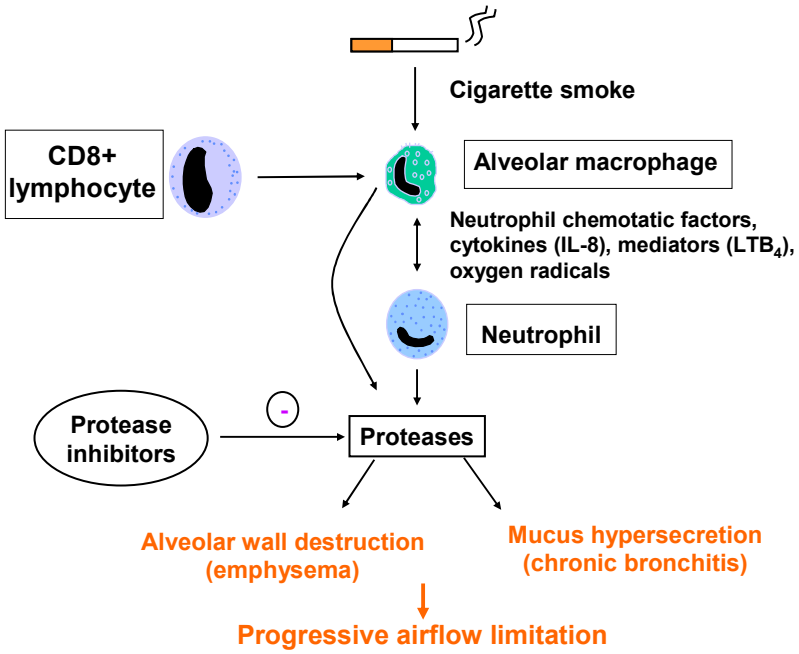


Figure 2.9 Disease processes in chronic obstructive pulmonary disease (Barnes 2000)

2.3.3 Diagnosis and assessment

A diagnosis of COPD should be considered in any individual presents with symptoms of chronic bronchitis, especially cigarette smoking together with confirmation of presence of airflow obstruction using spirometry. The hallmark of COPD is a reduction in the ratio of forced expiratory volume in one second (FEV_1) to forced vital capacity (FVC) to less than 70%. The degree of spirometry abnormality generally reflects the severity of COPD (NHLBI, 2004; GOLD, 2007). Although by definition COPD is an irreversible disease, reversibility testing is important in assessing both diagnosis, prognosis of COPD and determining treatment choice. Patients with an element of reversibility are those that respond to inhaled corticosteroids.

Classification of COPD by severity is as shown in Table 2.3.

Table 2.3 Classification of COPD by severity (Adapted from BTS, 2004)

Disease Severity	FEV_1 % predicted	Symptoms and signs
Mild	≥60	No abnormal signs Smoker's cough Little or no breathlessness
Moderate	40–59	Breathlessness (± wheeze) on moderate exertion. Cough (± sputum). Variable abnormal signs (general reduction in breath sounds, presence of wheezes)
Severe	<40	Breathlessness on any exertion/at rest Wheeze and cough often prominent Lung hyperinflation usually with cyanosis, peripheral oedema and polycythaemia in advanced disease, especially during acute exacerbations

2.3.4 Difference between asthma and COPD

Although asthma and COPD have similar characteristics such as the signs of coughing and wheezing, they are two distinct conditions in terms of disease onset, frequency of symptoms and reversibility of airway obstruction.

1. The onset of asthma typically occurs during childhood or adolescence (British Thoracic Society 2007). COPD most often develops in smokers and former smokers who are in their mid-50s (Petty 1995; Hogg 2004).

2. Exacerbations of asthma - characterized by recurrent wheezing, shortness of breath, chest tightness and cough - often have identifiable triggers such as allergens, cold air, exercise, viral infection or bacterial infection (British Thoracic Society 2007). However, exacerbations in COPD patients are commonly caused by respiratory tract infections (Pauwels et al. 2001).

3. With treatment the aim is for asthma patients to have near-normal lung function and be symptom-free between exacerbations (British Thoracic Society 2007). COPD patients rarely experience a day without symptoms. Airflow obstruction in COPD sufferers is only partially reversible (National Institute for Health and Clinical Excellence (NICE) 2004).

4. In COPD patients there are more neutrophils compared to patients suffering from asthma. In asthmatic patients the percentage of eosinophils is more compared to patients suffering from COPD. Since glucocorticoids are effective against inflammation caused by eosinophils (Altman et al. 1981) then these agents are useful in asthma. Inflammation that is mediated by the neutrophils is more resistant to the effect of glucocorticoids agents.

Smoking cessation decreases the accelerated downward progression of lung function and breathlessness whilst bronchodilator use provides symptom relief (British Thoracic Society 2007).

The maintenance therapy for most patients with asthma is an inhaled corticosteroid to control inflammation, with the addition of a bronchodilator, when required to control symptoms (British Thoracic Society 2007). However, the reverse is true for the treatment of COPD. Bronchodilators are the first line maintenance treatment for COPD. Treatment with inhaled corticosteroids is reserved only for selected patients whose COPD is not adequately managed with bronchodilators (British Thoracic Society 2007) that have moderate or severe COPD (FEV_1 50% predicted) and have frequent exacerbations (National Institute for Health and Clinical Excellence (NICE) 2004) A summary of the differences between COPD and asthma are shown in Table 2.4.

Table 2.4 Differential diagnosis for chronic obstructive pulmonary disease and asthma.

History	COPD	Asthma
Smoker or ex-smoker	Nearly all	Possibly
Symptoms under age 45	Rare	Often
Chronic productive cough	Common	Uncommon
Breathlessness	Persistent and progressive	Variable
Night wakening with breathlessness and or wheeze	Uncommon	Common
Significant diurnal variation or day to day	Uncommon	Common

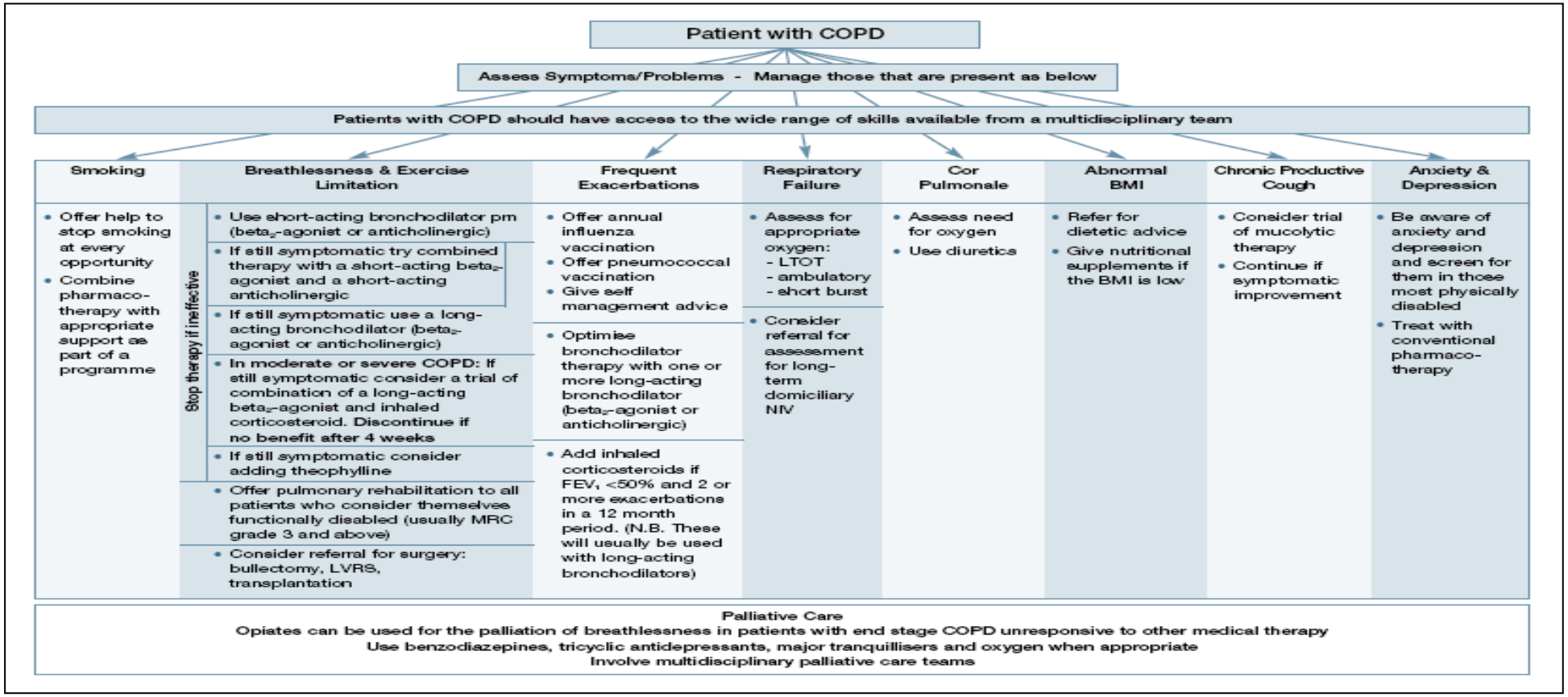
2.3.5 Management of stable COPD

The goals of management of COPD are to:

- Enable early and accurate diagnosis
- Control symptoms
- Prevent deterioration
- Prevent complications
- Improve quality of life

Although by definition COPD is an irreversible disease, reversibility testing is important in assessing diagnosis, prognosis of COPD and determining treatment choice. Hence the results of reversibility tests are important for future management, they should be clearly documented and be easily available for future reference. A summary of National Institute for Clinical Excellence (NICE) recommendation gives a broad outline to treat and stabilise COPD. A schematic design of the NICE guidelines recommendation for the therapeutic management of patients with stable COPD is described in Table 2.5.

Table 2.5 Summary of the recommended managements of stable COPD [reproduced from (NICE 2004)]



2.3.5.1 Smoking cessation

All COPD patients should be encouraged to stop smoking as this is the most effective way to improve outcomes and prevent further accelerated airway obstruction (Fletcher and Peto 1977; National Institute for Health and Clinical Excellence (NICE) 2004).

Table 2.5 Summary of the recommended managements of stable COPD [reproduced from (NICE 2004)].

2.3.5.2 Bronchodilators

All COPD patients should be given an inhaled bronchodilator to provide relief of symptoms (Celli et al. 2004). If they help the patient to perform normal daily activities or to improve exercise tolerance, it is worthwhile continuing this treatment (National Institute for Health and Clinical Excellence (NICE) 2004).

Inhaled short-acting β_2-agonists (e.g. salbutamol, terbutaline) have a relatively rapid onset of action and are often used as required to relieve symptoms (National Institute for Health and Clinical Excellence (NICE) 2004). Inhaled antimuscarinics (e.g. ipratropium bromide) are as efficacious as short-acting β_2-agonists in COPD and may provide a greater and longer bronchodilator response. However, due to their slower onset of action, antimuscarinics may be less suitable for symptom relief than β_2-agonists.

Clinical trial evidence also recommends the use of long-acting β_2-agonists (e.g. salmeterol and formoterol) in COPD. Long-acting β_2-agonists have a prolonged duration of action from 12-14 hours. In addition to their bronchodilator action, long-acting β_2-agonists also inhibit mast cell mediator release, plasma exudation and may reduce sensory nerve activation (Nials et al. 1994). Long-acting antimuscarinic bronchodilators show muscarinic M_1 and M_3 receptor subtype selectivity. Tiotropium bromide, the first of a new class of selective and long-acting antimuscarinic agents was introduced for once daily maintenance treatment of COPD patients. The combination of long-acting β_2-agonists and tiotropium

bromide exhibited additive effects in terms of daytime lung function improvements and sustained improvements during the night compared with the single components, despite the once daily dosing (Cazzola et al. 2004; Cazzola et al. 2005; van Noord et al. 2005). The NICE / BTS guidelines recommend that the combination of two long-acting bronchodilators with different pharmacological mechanisms of action should be considered in all patients with moderate to severe chronic obstructive pulmonary disease. The rational for using both of the anticholinergics and the β_2-agonist is that the anticholinergics will inhibit the vagal tone that exists in COPD. This will help the airways to relax so when the β_2-receptors are stimulated the bronchodilatation should be enhanced. A combination treatment of tiotropium and formetrol was more effective than the single agents with respect to bronchodilation in COPD patients (Cazzola et al. 2004; Cazzola et al. 2005; van Noord et al. 2005). These two studies highlighted the value of using a long acting β_2-agonist together with a long acting anticholinergic agent.

There is limited evidence showing the benefit of theophylline in COPD. Theophylline may, however, cause serious side effects which may occur within the normal dosage range. The use of theophylline is therefore not strongly recommended and limited to those in whom other treatments have failed to control symptoms. Mucolytic therapies have recently had a lot of interest and should be considered in patients with a chronic productive cough. The aim of treatment is to reduce the frequency of cough and sputum production. A meta-analysis of such agents revealed that when used for more than two months there is a reduction in exacerbations by 29% correspond to placebo (Poole and Black 2001).

2.3.5.3 Corticosteroids

The pathogenesis of airway obstruction in COPD is multifactorial, involving neutrophilic airway inflammation (Stanescu et al. 1996), protease-antiprotease imbalance (Tetley 1993), oxidative stress (Repine et al. 1997), and recurrent infection. These mechanisms are interrelated such that reducing one factor may also reduce the stimulus to others.

An increased number of neutrophils are present in the lungs of cigarette smokers compared with that in non-smokers. Cigarette smoke may attract neutrophils to the lung by stimulating alveolar macrophages to release a potent chemotactic factor for neutrophils (Hunninghake and Crystal 1983). These increased neutrophils are associated with a rapid decline in the FEV_1 (Stanescu et al. 1996). Furthermore, neutrophils activation markers are elevated in the sputum supernatants of subjects with COPD (Keatings and Barnes 1997), suggesting that neutrophils are active participants in airway inflammation. There is still an ongoing debate about the benefit of inhaled or oral corticosteroids in patients with stable COPD. While corticosteroids have no effect on inflammation caused by neutrophils, they may also influence the cyctokine level (Keatings et al. 1996).

Inhaled budesonide was found to be of no clinical benefit in COPD patients recruited from the general population by screening (Vestbo et al. 1999). Although the ISOLDE study (Burge et al. 2000) showed benefits from inhaled fluticasone, the TORCH (Towards a revolution in COPD Health) study (Calverley et al. 2003) did not. Only those COPD patients with a positive response to a corticosteroid reversibility test should be considered for inhaled steroid therapy without long-acting β_2-agonists. It has also been noted that the combination of corticosteroid and long-acting β_2-agonist in a single inhaler improved lung function and decreased severity of dyspnoea in patients with COPD (Cazzola and Dahl 2004). The TORCH study has also published and consolidated these findings (Calverley et al. 2003). A similar study by Szafranski, et al., (2003) has shown the benefit of budesonide in combination with formoterol. The NICE guidelines suggest that if a patient is prescribed

a long-acting β_2-agonist and has a FEV_1 <50% with two or more exacerbations per year then a high dose of inhaled corticosteroid with a long-acting β_2-agonist should be considered. Recently, from the results of the TORCH study, the prescription licence for Seretide (Fluticasone and Salmeterol) has been changed to allow those with a FEV_1 <60% to be prescribed this inhaled combination.

Also the benefit of the combination of a long acting β_2-agonist and an inhaled corticosteroid in a MDI was demonstrated in a study by Theophilus, et al., (2006). They demonstrated that there is a significant co-association of salmeterol and fluticasone propionate particles, leading to increased co-deposition when they are administered from the same inhaler. This provides a greater opportunity for a synergistic interaction between the two drugs to occur in the airways (Barnes et al. 2006) and may possibly be a significant factor contributing to the enhanced clinical effect seen in comparison with that observed when the drugs are administered separately from two inhalers (Theophilus et al. 2006).

COPD patients may develop Cor Pulmonale (a secondary heart disease) with pulmonary hypertension, right ventricular hypertrophy and right heart failure. Patients with COPD frequently suffer acute exacerbations of their symptoms, and may require hospitalization (Vestbo et al. 1999). Treatment options include antibacterial agents and oxygen as necessary, together with appropriate managements of any associated cardiovascular disorder (Vestbo et al. 1999; Martindale 2002; Hogg 2004).

2.3.5.4 Supplemental long-term oxygen therapy (LTOT)

Supplemental long-term oxygen therapy (LTOT) improves survival, exercise, sleep and cognitive performance in hypoxaemic patients (Eaton et al. 2004; Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2007). Arterial blood gas (ABG) assessment is the preferred method to determine oxygen need because it includes acid-base information. Arterial oxygen saturation as measured by pulse oximetry (SpO_2) is used as well in determining oxygen need. Physiological indications for oxygen include an arterial oxygen

tension (PaO_2) <7.3 kPa (55 mmHg). The therapeutic goal is to maintain SpO_2>90% during rest, sleep and exertion to prevent tissue hypoxia. If CO_2 retention occurs, it is suggested to monitor for acidemia. If acidemia occurs, mechanical ventilation is essential for the survival of the patient.

2.4 Inhaler devices

Successful management of asthma and other lung diseases depends on achieving adequate delivery of inhaled drug to the lungs and it is generally acknowledged that the inhaler device is a key element in determining the success (Selroos et al. 1996). Ideally, an inhaler device should be easy to use and able to deliver a predetermined dose of drug to the lung in a reproducible and cost-effective manner. The three types of inhalers are: nebulisers, pressurised meter dose inhalers (MDIs) and dry powder inhalers (DPIs).

2.4.1 Nebulisers

These are devices that convert a solution or suspension of drug into aerosol droplets suitable for inhalation. Although nebulisers can be used in patients who cannot master the correct use of a MDI or DPI (Fink 2000), there are most commonly used where the therapeutic dose is too large for delivery using the alternative systems (Taylor and McCallion 1997). Generally, nebulisers are bulky, costly and more complex. Indications for nebulised treatment in asthma have declined, especially in community settings (BTS in Thorax 1997). Concentrated solutions are used in nebulisers from which aliquots are withdrawn and diluted before administration. Some of these solutions contain preservatives and antioxidants which sometimes cause bronchospasm. The physicochemical properties, e.g. viscosity and surface tension, of the nebulised solution significantly affect the performance of nebulisers (Taylor and McCallion, 1997). Two types of nebulises are available: Jet nebulisers and Ultrasonic nebulisers.

2.4.1.1 Jet nebulisers

These utilise compressed gas from an air or oxygen cylinder or electrical compressor to convert a drug solution into a spray. The compressed gas passes though a narrow venture orifice at a high velocity which causes the liquid from a fluid reservoir to disperse into droplets. Smaller droplets leave the nebuliser directly and can be inhaled.

2.4.1.2 Ultrasonic nebulisers

These utilise mechanical energy produced by a piezoelectric transducer that usually vibrates to generate the spray.

2.4.2 Metered dose inhalers (MDIs)

Metered dose inhalers were introduced in the 1950s and remain the most popularly used inhaled products (Vaswani. and Creticos 1988). MDIs consist of a canister containing the drugs dissolved or suspended in a propellant e.g. chlorofluorocarbons (CFC) and moe recently hydrofluoroalkanes (HFA) under pressure. When activated, a valve system releases a metered volume of drug and propellant. The propellant provides the force to release and de-aggregate particles. The fast release of the particles causes high oropharyngeal deposition and produces a cooling sensation at the back of the throat, known as cold Freon effect (Fink, 2000) that may stop some patients inhaling immediately after actuation thereby depositing the large portion of the dose in the mouth. MDIs require co-ordination between the start of an inhalation and release of the dose from the device, which many patients; especially children and elderly find difficult (G.K.Crompton 1982). The ban on the use of chlorofluorocarbons (CFC) propellant in MDIs to protect the ozone layer (Montreal Protocol 2000) has led to the development of MDIs containing hydrofluoroalkanes (HFAs), non-ozone depleting propellants that replaced CFC-MDIs. The change to HFAs propellants has generated formulation issues with changes in the size and the velocity of the emitted droplets.

2.4.3 Spaces and valved holding chambers

These are add -on devices, which used properly, reduce oropharyngeal deposition of drug, increase the lung deposition, eliminate the cold Freon effect and in case of valved holding chamber, reduce drug loss associated with poor patient coordination (Vaswani.S.K. and Creticos 1988; Fink 2000; Aswania and Chrystyn 2001; Terzano 2001). However, spacers have the disadvantage of being bulky to carry and patient compliance with therapy could be decreased (Keller.M. 1999).

2.4.4 Dry powder inhalers (DPIs)

Dry powder inhalers basically contain four functional elements: the powder container, the metering system, the disintegration principle and a mouthpiece. Based on these functional elements, dry powder inhalers can be divided into two major groups, single dose and multi-dose inhalers (Table 2.6).

Table 2.6 Dry powder inhalers available in the market

Inhaler device (manufacturer)
Single dose inhalers
Spinhaler (Aventis)
Cyclohaler (Pharmachemie)
Rotahaler (GlaxoWellcome)
Aerolizer (Novartis Pharma)
Inhalator (Boehringer Ingelheim)
Handihaler (Boehringer Ingelheim)
Multi-dose inhalers
Multiple unit-dose inhalers
Diskhaler (GlaxoWellcome)
Aerohaler (Boehringer Ingelheim)
Diskus / Accuhaler (GlaxoWellcome)
Reservoir systems
Turbuhaler (AstraZeneca)
Clickhaler (Innovata Biomed/ML labs celltech)
Easyhaler (Orion Pharma)
Pulvinal (Chiesi)
Novolizer (Viatris)

For the marketed dry powder inhalers, only two different types of powder formulations are currently used: spherical pellets and adhesive mixture. Spherical pellets are used in the Turbuhaler. In this type of formulation, the micronized drug particles are agglomerated

into much larger spherical units without a binding agent, behaving as a free flowing powder. Some micronized diluents such as lactose or glucose may be added to the active component when the dose is low (e.g. Formoterol 6 and 12μg), but the formulation does not contain coarser carrier crystals. Spherical pellets have to disintegrate nearly completely during inhalation into much smaller agglomerates or even primary particles that have the required size-range for deep penetration into the respiratory tract.

All other DPIs are filled with adhesive mixtures. This type of formulation consists of relatively large carrier crystals, mostly α-lactose monohydrate, carrying the micronized drug particles distributed over their surface. During inhalation, the drug particles have to be released from the carrier crystals to generate the aerosol with particles of the desired particle size, which are able to enter the lower respiratory tract.

The principle of operation for a DPI is to use the patient generated inspiratory flow as an energy source for the release of the dose and the delivery of fine drug particles into the respiratory tract. The particle size of the adhesive mixtures or the spherical pellets is far too large for lung deposition. Therefore, the pellet or mixture has to be disintegrated to make an aerosol cloud, which contains a high fraction of non-agglomerated drug particles with the desired particle size (<5 μm). Many different disintegration principles exist. They may vary from a simple screen (Rotahaler, Diskhaler), to twisted powder channels (Turbuhaler). The applied disintegration concept in the design of a dry powder inhaler largely determines the resistance to airflow of the inhaler device.

Inhalers without a recognisable disintegration principle (Figure 2.10) such as Diskhaler and Accuhaler, often have a low (medium) resistance to airflow.

Inhalers with specific disintegration systems like the Turbuhaler use inspiratory flow more optimally as the energy source for disintegration and delivery of fine particles into the airflow (Figure 2.11). This usually results in an increased resistance to airflow through the inhaler.

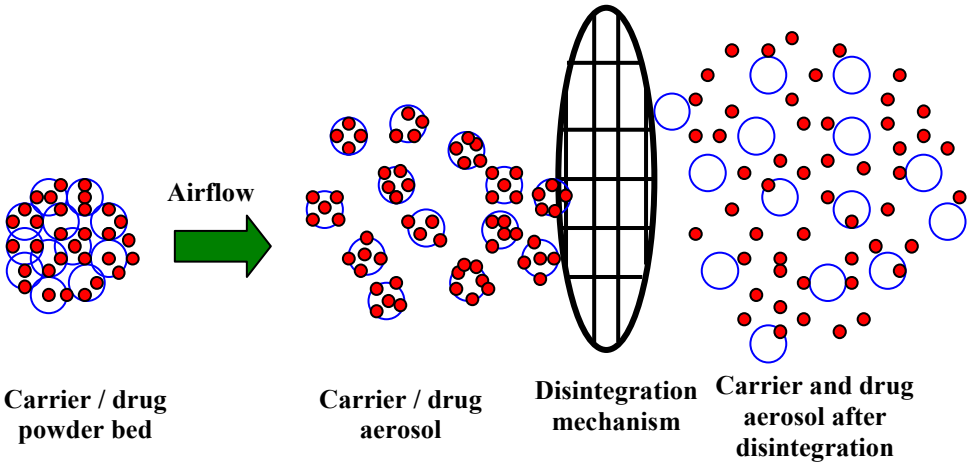


Figure 2.10 Schematic diagram of the disintegration of micronized drug particles from carrier crystals through a non-specific disintegration system.

The peak inspiratory flow achieved by a patient through a powder inhaler depends upon the specific resistance to flow of the device and the patient inspiratory capability for a set of inspiratory effort. The pressure change that occurs across the mouthpiece on inhaler during an inhalation represents the turbulent energy inside the inhaler device that uplifts and de-aggregates powder formulation into fine particles with the required size <5µm. This turbulent energy is related to inhalation flow and airflow resistance in the device as described in section 2.5.2.2 of this thesis. The lower the specific resistance in the DPI the higher will be the peak inspiratory flow (Clark and Hollingworth 1993). Therefore, resistance to airflow is one of the design parameters for DPIs that could be used to control the inspiratory flow profile and optimize particle deposition in the airways (de Koning et al. 2002). Thus the more resistance there is inside the inhaler then the lower will be the inhalation flow for a set inspiratory effort. The high resistance to airflow limits the range of possible inhalation flows. However, due to the higher disintegration efficiency, the fine particle output is higher compared to the non-specific disintegration systems (Srichana et al. 1998; Hawksworth et al. 2000; de Koning et al. 2002; Srichana et al. 1998). The mouthpiece may be used to control the resistance to airflow of the inhaler and the direction

of the aerosol cloud in the mouth and throat, in order to reduce drug deposition in the oropharyngeal cavities (de Boer et al. 1997).

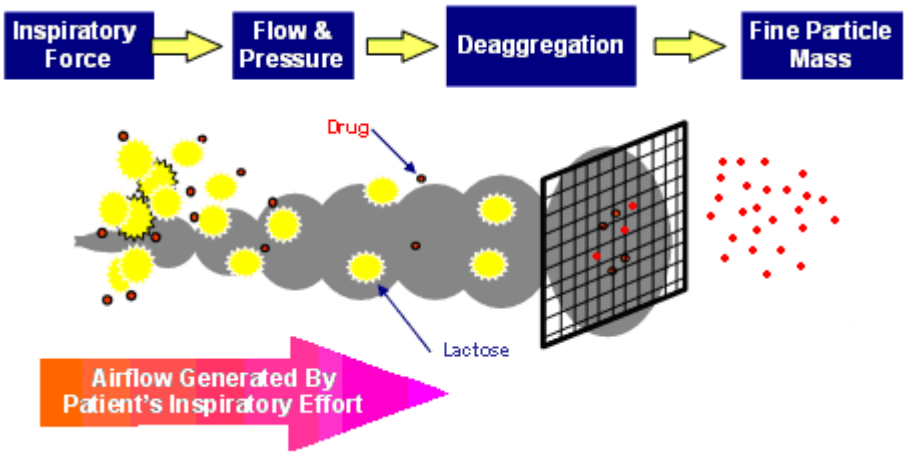


Figure 2.11 Schematic diagram of the disintegration of spherical pellets through a specific disintegration mechanism [Reproduced from (Chrystyn 2003)].

Some advantages and disadvantages of dry powder inhalers are summarised in Table 2.7.

Table 2.7 Advantages and disadvantages for dry powder inhalers versus metered dose inhalers (Ashurst et al. 2000).

Advantages of dry powder inhalers	Disadvantages of dry powder inhalers
• Propellant free	• Performance depends on the patients inspiratory flow profile
• Less need for patient co-ordination	• Resistance to airflow of the device
• Less potential for formulation problems	• Potential difficulties to obtain dose uniformity
• Less potential problems with drug stability	• Less protection from environmental effects and patient abuse
• Less potential for extractable from device components	• More expensive

2.4.4.1 Single unit dose dispensing system

The early DPIs were all unit dose systems such as the Spinhaler® which was introduced in 1969 and the Rotahaler® in 1977. As single dose inhalers, both utilize pre-metered doses dispensed into hard gelatine capsules with a different mechanism of powder delivery. The capsule cap and body must be separated before inhalation (Rotacaps® for Rotahaler®) or the capsule has to be pierced at both ends; as for the capsules for the Aerolizer®, the Spincaps ®for the Spinhaler®, and the Spiriva® Handihaler.

Other devices in this category are the Handihaler®, Berotect® (Bohringer) and Forodil Aeroliser®. The main disadvantages of these devices are the loading procedure which may be difficult to achieve by a patient with an asthma attack and possible requirement to make two inhalations for each dose.

2.4.4.2 Multiple unit dose dispensing system

The multi-dose inhalers are divided in two different types of design: the reservoir systems and the multiple unit-dose inhalers. The reservoir-system of DPI includes the Turbuhaler, the Clickhaler®, the Pulvinal®, the Novolizer® and the Easyhaler®. In this type of inhaler, the powder formulation is stored in a reservoir from which single doses are measured volumetrically and dispensed with a special dose metering unit. Accurate dose metering for this type of inhaler requires careful manipulation of the device by the patient. In the multiple unit-dose inhalers, single doses are filled by the manufacturer into suitable dose compartments, such as blisters. Examples are Diskhaler®, having the blisters on a disk (Rotadisk), and Accuhaler® in UK) with the blisters on a long strip.

(a) Diskhaler®

This device has individual doses contained within a blister on a disk. On priming the device, the blister is pieced on both side and the powder is ready to join the inhalation stream.

(b) Accuhaler® (Diskus®)

The Accuhaler® (Diskus®) as shown in Figure 2.12 contains sixty factory dispensed doses each in a blister and sealed. The formulation is not exposed to the environment. The seal is opened immediately before use by sliding back the lever. The mouthpiece opens once the blister is unsealed, and the dose is ready to be inhaled. If the dose is not inhaled at this point, closure of the device will empty the dose into a collecting chamber within the device and stored away from the inhalation channels. Most inhaled drugs for management of asthma and chronic obstructive pulmonary disease (COPD) are available in the Accuhaler dry powder inhaler. These are the short and long acting ß2-agonists (salbutamol and salmeterol), a corticosteroid (fluticasone) and a combination of a long acting ß2-agonist and corticosteroid salmeterol and fluticasone (Seretide). The Accuhaler has a dose counter that shows the number of doses left in the device after each actuation, which prevents inadvertent double dosing.

.

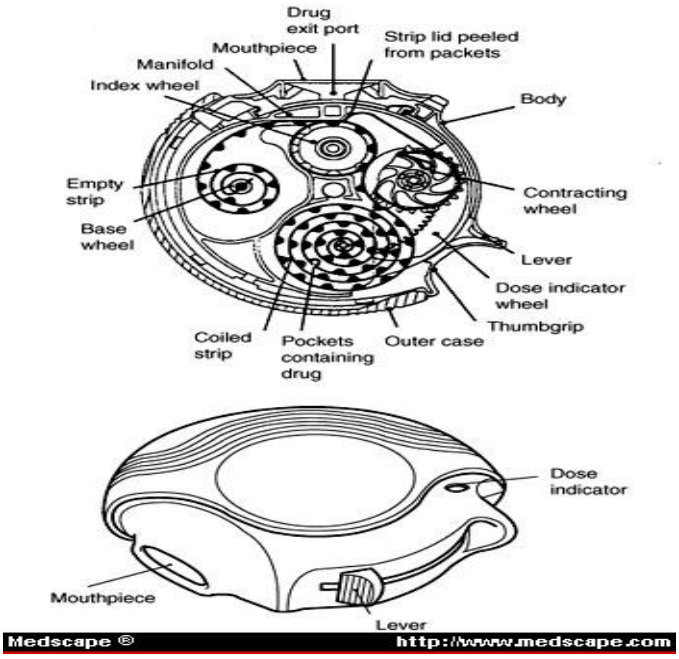


Figure 2.12 Accuhaler® from (Boulet et al. 1995)

2.4.4.3 Multiples doses dispensing system

(a) Turbuhaler®

The Turbuhaler is a reservoir multidose dry powder inhaler containing 200 doses. The device has been designed to deliver small quantities of the pure drug as spherical pellets/beads, which shatter during inhalation. It consists of the following parts:

(1) Mouthpiece with insert (2) Bypass air inlet (3) Inhalation channel (4) Air inlet (5) Desiccant store (6) Window for dose indicator (7) Dose indicator (8) Storage unit for drug compound (9) Dosing unit (10) Operating unit (11) Turning grip.

A simple twist (back and forth) of the base with the device held upright meters each single dose. During inhalation the air enters through the inlets and passes through the unit to release the dose that has been loaded in the conical holes within the inhalation channels. De-aggregation of the drug particles takes place in this channel due to the air turbulence that is created during the inhalation process. The Turbuhaler has a dose indicator, which shows red in the window of the dose indicator when there are 20 doses left.

The device delivers carrier free particles of either the β-agonist terbutaline sulphate or salbutamol sulphate (not available in the UK) and corticosteroid budesonide. It is also available as Symbicort containing budesonide and formoterol. More recently the Mark3 version of the Turbuhaler Polmucult (containing the combination of budesonide and eformoterol has a dose counter). The Turbuhaler has been designed to create turbulent energy by using a long flow path with spiral channels to generate shear forces that disperse the drug aggregate and produce the required fine particle dose (K.Wetterlin 1998). However, due to this long flow path, the resistance within the device is high and the device typically delivers only 60% of the metered dose as result of the greater losses within the device (Byron 1990). Patients using the Turbuhaler have to use a significantly higher

inspiratory effort compared to other DPIs in order to achieve the same inspiratory flow inside the device (Celga 2004).

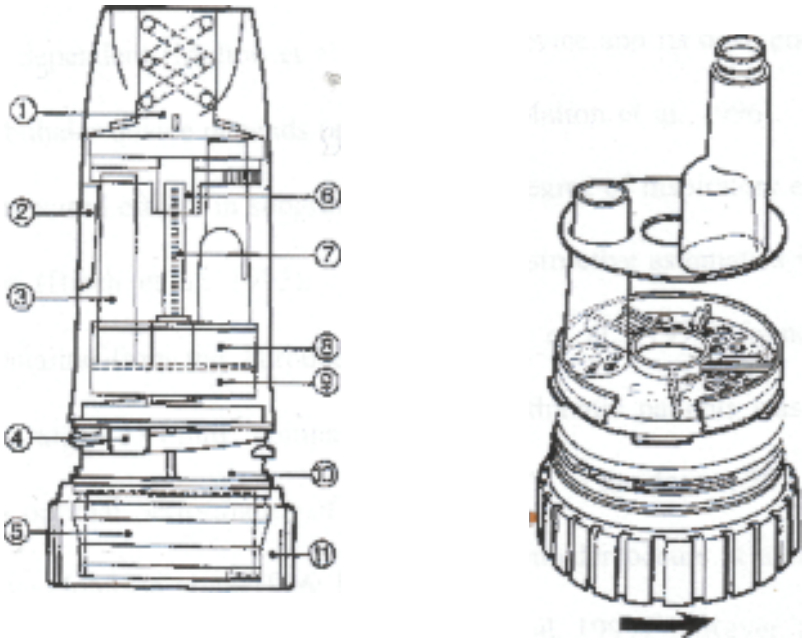


Figure 2.13 Turbuhaler (adapted from Watterlin, 1998)

(b) Easyhaler®

The Easyhaler® (Figure 2.14) is a multiple dose dispensing system that contains 200 doses. It is a high resistance device containing a mixture of a drug and lactose. The mixture is stored in a hopper at the bottom of the device; a dosing cup on a rotating drum is filled with drug powder. Shaking the inhaler ensures an even dispersion prior to dose metering. While the device is upright, and by pressing the top of the device dosing cup deposits the drug and carrier into the inhalation channel where turbulent airflow breaks up the mixture into fine particle dose, which is then deposited into the patient's lungs during inhalation. Double dosing cannot occur with the Easyhaler because the mechanism and counter allow patients to see if they loaded a dose to prevent the use of empty device. Also if a dose is

not inhaled and another is metered the unused dose is deposited inside the inhaler away from an inhalation airstream.

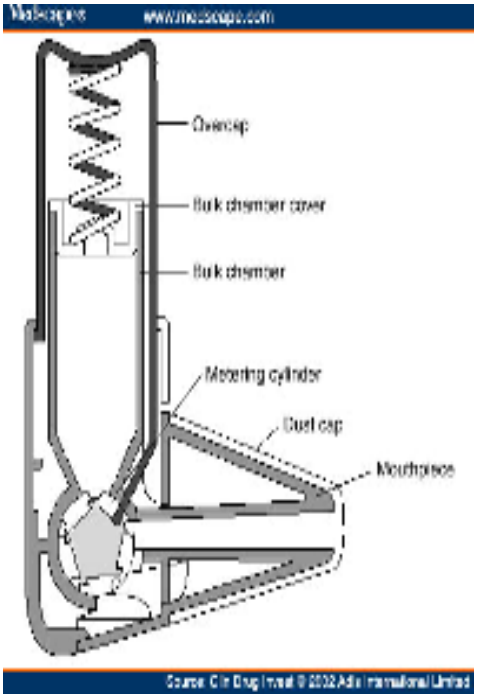


Figure 2.14 Easyhaler-a multidose device containing pre-loaded drug doses within a powder reservoir

(c) Clickhaler®

The Clickhaler® is a reservoir device that delivers 200 doses. The device is primed by clicking the button on the top of it with the inhaler in an upright position (Figure 2.15). This causes the metering cone on a rotating wheel to take a single dose from the drug hopper and eventually the loaded dose reaches the inhalation passage and the dose is ready to be inhaled. The safety of the device include the prevention of inadvertent double dosing, a dose counter on the back of the device and a lock-out after 200 doses.

Figure 2.15 Clickhaler (adapted from www.vectura.com/vec/images/clickhaler

2.5 Pulmonary drug delivery

The success of inhaled therapy depends on the ability to deliver adequate aerosolised drug in optimal size range to appropriate sites in the lungs with minimal side-effects. The advantages of inhaled therapy compared with other forms of therapy include ease of administration, topical delivery of minute but effective doses to therapeutic active sites, rapid action, avoidance of the gastrointestinal upset from oral therapy and the first pass effects in the intestine and liver (Barnes, 2004; Chrystyn, 2006b). The surface area of bronchioles and alveoli facilitates the rapid absorption of the inhaled medicament and this may additionally be employed as a route for delivery of drugs into the systemic circulation. The type of drug formulation and device as well as the intra and inter patient variability and inhalation technique are important factors in considering drug delivery and deposition to the respiratory system.

2.5.1 Mechanism of particle deposition in the lungs

Deposition of aerosolised drug in the respiratory tract is a complex interplay of aerodynamic behaviour of aerosol particles and the anatomy and physiology of the lungs. It occurs as a result of three principal mechanisms: inertial impaction, gravitational sedimentation, and Brownian motion (Lippman et al., 1980).

The first mechanism is **inertial impaction** which is the dominant deposition mechanism for particles in the upper respiratory tract (mouth, pharynx, larynx, and tracheobronchial region). A particle with a large momentum (the product of velocity and mass) carried in inspired air is unable to follow the changing direction of the inspired air as it passes the bending and branching of the upper respiratory tract. This large momentum leads to impaction on the airway walls. The probability of impaction is dependent upon the momentum, thus a particle with a large diameter or high density travelling in the airstreams at higher velocity will show greater impaction. The airflow velocity in the main bronchi is estimated to be 100-fold higher than that in the terminal bronchioles, and 1,000 fold higher than in the alveolar region (Hillery et al. 2001). Large particles with aerodynamic diameter greater than 5μm, will deposit in the mouth and oropharyngeal region and are swallowed (Svartengren et al. 1991).

The second mechanism of deposition is **sedimentation**. The smaller particles do not impact and are carried by the inspired air into the conducting airways. As the pulmonary tree branches into smaller diameter conducting airways, particles between 2 and 5μm deposit in these airways (tracheobronchial region) by impaction and sedimentation.

As the inspired air containing particles moves further down the bronchial tree the velocity of air stream becomes slower and slower. Particles between 0.5 and 2μm are suspended in slow moving air of lower airways and deposited by gravitational sedimentation. This occurs in the bronchioles and the alveolar region where the airflow is low. The fraction of particles deposited by this mechanism will be dependent upon the time the particles spend in these regions. Holding the breath after an inhalation increases the time the particles spend in these regions thus increases deposition by this mechanism (Hyder 1982; Newman and Clarke 1983; Hillery et al. 2001).

The third principle mechanism of deposition is **Brownian diffusion**. This usually occurs for particles with a particle size lower than 1μm, as particles below this size are displaced

by random bombardment of gas molecules, which results in particle collision with the airway walls. The probability of particle deposition by diffusion increases as the particle size deceases and it is also more prevalent in regions where airflow is very low or absent, e.g. in the alveoli. Holding the breath after an inhalation increases deposition by this mechanism. Figure 2.16 shows a diagram of the above mentioned three mechanisms (Hillery et al. 2001). The majority of these very small sized particles do not deposit in the lungs (Lippmann et al. 1980).

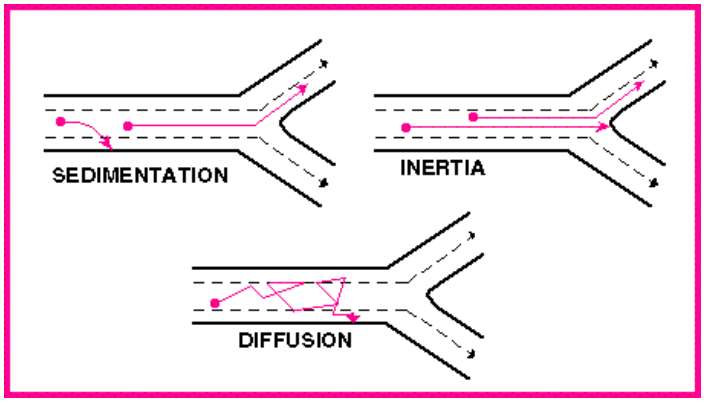


Figure 2.16 Particle deposition mechanisms at airway branching site.

2.5.2 Factors affecting deposition of aerosol particles

2.5.2.1 Aerosol particle characteristics

The aerodynamic particle size is potentially the most important factor determining the site of aerosol deposition. Deposition throughout the conducting airways of the lungs is necessary for effective response following inhalation of ß2-agonists and corticosteroids. This is achieved by using particles of an aerodynamic diameter between 2 and 5µm (Chrystyn, 1999), during an inhalation. Particles greater than 5µm in aerodynamic diameter most likely to be deposited in the upper including the mouth, nose and pharynx thus eventually swallowed. For particle size 0.5-2 µm in diameter, most of the aerosol is deposited in the alveoli by Brownian motion with gravitational sedimentation as previously described. Anti inflammatory agents that deposit in this area may exert some therapeutic effect. Studies by (Cartairs et al. 1989) and (Barnes 1995) have demonstrated that

receptors of ß2-agonists are situated from the trachea down to the terminal bronchioles. ß2-agonists dilate airways by their action on smooth muscles ß2-receptors but there is doubt about their effects on alveolar sacs. A study in which 10 asthmatic patients inhaled terbutaline, there was little bronchodilation when the formulation containing drug particles of >5 µm diameter were inhaled. Particles above 5 µm in diameter have most likely been deposited in the mouth and throat (Rees et al. 1982). Another study on ten asthmatic patients using 20 µg of salbutamol from specially prepared MDI consisting monodispersed particles with mass median aerodynamic (MMAD) of 1.2, 2.8 or 5 µm has shown that the bronchodilator responses with particles of 2.8 µm was the best. These responses were slightly better for the 5 µm particles compared to 1.5µm (Zanen et al. 1996). This implies greater peripheral deposition in the alveoli with the smaller particles and because there are no smooth muscles in this area, these airways are less liable to dilate. These suggest that, in mild asthma, the particle size of choice for a ß2-aerosl should be close to 2.8 µm. It has been suggested particularly for patients with obstructive lung diseases, all particles should ideally be within the 2-3µm range (Terzano 2001).

All currently available DPIs rely on the inspiratory effort of the patient to lift the powder formulation from the drug reservoir, the dosing disk, the blister or capsule. The inspiratory effort generated by the patient also disaggregates the powder into particles small enough with the greatest potential to reach the therapeutic sites in the lungs as previously described in Figure 2.11. A more forceful inhalation through a powder inhaler will result in better disaggregation, finer particles and a higher amount of drug reaching the lungs which may be beneficial in the management of asthma or chronic obstructive pulmonary disease (Borgstrom et al., 1994).

Therefore, it is important that patient have sufficient inspiratory capacity and can use their inhaled medication according to the instructions provided by the manufacturer.

2.5.2.2 Inhalation technique

The site of aerosol deposition in the lung is dependent on the patient's inhalation technique, i.e. the way in which the patient uses an inhaler. This entails an interaction between the inhaler-formulation and the patients inhalation manoeuvre which include the volume of air inhaled, the inhalation flow, the breath holding period after inhalation and the volume of the lungs at the initiation of the inhalation that contribute to successful delivery and deposition of aerosol to the lungs of patients, and thus enhancing efficacy of inhaled therapy (Timsina et al. 1994). Figure 2.17 describes how the relationship between the patient and the inhaled product provides effective asthma/COPD management.

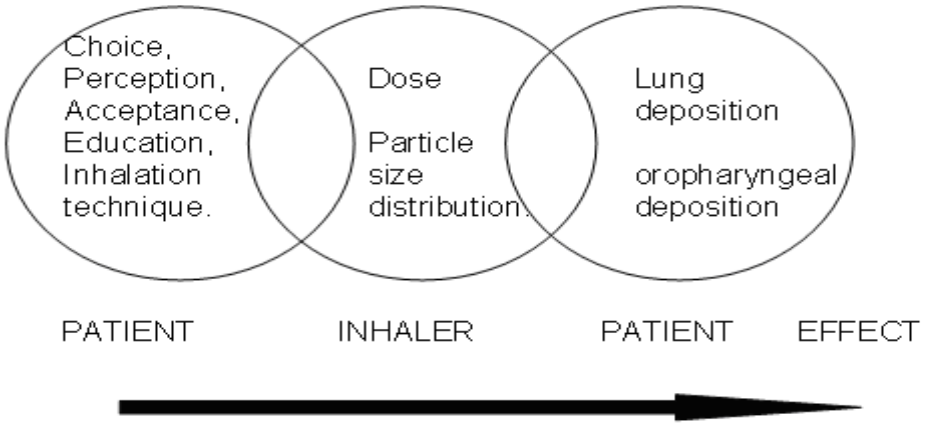


Figure 2.17 The relationship between the patient and the inhaled product (adapted from Chrystyn not published)

Generally, for particles that deposit in the airways by sedimentation mechanism (i.e., particles > 0.5 and < 3.0 μm), an increase in inhalation volume and the use of breath-hold will enhance deposition in the peripheral airways as a result of increasing residence time, therefore, subjects using inhaler devices are instructed to take deep breath, in order to distribute particles to the periphery of the lungs (Newman and Clarke 1983).

Although a large volume of inhalation is desirable, a fast inspiratory flow is not for MDIs as more deposition occurred in the mouth and throat (Newman et al. 1981.). The lung

volume at which an aerosol is actuated is also important. (Newman et al. 1982) found that deposition is enhanced if the aerosol is released during the early stages of a slow inhalation. This means that for an MDI, a slow and deep inhalation. This study showed that co-ordination is not important as long as the patient uses a slow inhalation and actuation after the start of the inhalation.

For dry powder inhalers (DPIs), the most important factors affecting the dose delivery are the peak inspiratory flow of the patient, acceleration rate, inspiration time, resistant to flow of the device, and formulation in the inhaler (de Boer et al. 1996). Dry powder inhalers (DPIs) rely on the patient's inhalation flow (especially the initial acceleration of the inhalation flow within the device) for drug delivery to the lungs. The patient's inhalation flow interacts with the resistance inside the DPI to generate a turbulent energy which de-aggregates the formulation into an emitted dose containing particles that have the potential for lung deposition (Chrystyn, 2003). The part of the emitted dose from an inhaler device with particles in size range (<5μm) that have the greatest potential to deposit in the lungs is known as fine particle dose. Since the dose leaves its metering cup, inside the DPI, during the first few milliseconds of the inhalation manoeuvre then the fast inhalation should occur immediately. Failure to achieve a fast inhalation at the beginning (acceleration rate) results in the emission of particles that are too big to be deposited into the lungs and so these are only deposited in the mouth (Everard et al. 1997). A DPI should be inhaled with a fast suck that is as deep and hard as possible.

All DPIs have a different internal resistance (Chrystyn, 2003b) that decreases the inhalation flow used by a patient. The turbulent energy inside a DPI is represented by a pressure change ($\sqrt{\Delta P}$) that developed across the device during inhalation. This pressure change is directly related to the DPIs` internal resistance to airflow (R) and the inhalation flow (Q) and the relationship is described as: $\sqrt{\Delta P}$ = QR (Clark and Hollingworth 1993). Since the turbulent energy is a product of the flow and the inhaler's resistance then for a

set energy level (inspiratory effort) the flow required through a low resistance DPI will be faster than that of a high resistance DPI. The faster the inhalation flow through a DPI then the greater will be the turbulent energy and therefore the better is the quality of the emitted dose, i.e. aerosol with finer particles. Hence all DPIs have flow-dependent dose emission with some DPIs more prone to this than others (Chrystyn, 2003c). There is a minimum threshold energy required at which the de-aggregation is sufficient to provide a dose with the potential to produce particles with the required size. It is generally accepted that this minimum threshold energy is equivalent to an initial inhalation flow of 30 $Lmin^{-1}$ through a DPI. This minimum threshold comes from Turbuhaler® studies (Bisgaard, et al., 1998; Newman, et al., 1991). Therefore, similar values would apply for DPIs with a similar resistance to the Turbuhaler® such as a Clickhaler®. DPIs with a higher resistance (Easyhaler®) would require a lower flow whilst for those with a lower resistance (Accuhaler® and Novolizer®) would require a faster inhalation flow (Assi and Chrystyn 2001).

Studies have highlighted that some patients have problems achieving a fast rate during routine use with a DPI (Chrystyn, 2003; Pedersen et al. 1986; Pedersen and Steffensen 1986). These studies have revealed that young children and those with severe obstruction are most likely to have problems using a fast inhalation flow. Since DPIs are very much dependent on achievement of a certain inspiratory flow, then there is a risk of reduced effects during episodes of acute wheeze or in patients with low pulmonary function (Pedersen 1987). Since patients will inhale at a different flow then the design and formulation of each inhaler should be such that the most desirable range of inhalation flow required is achievable by all patients, of all ages and at all times.

The literature highlighted a link between inhalation flow, emitted dose (fine particle dose), lung deposition, and clinical response.

(a) In-vivo flow dependent dose emission.

Many studies have used gamma scintigraphy to highlight that lung deposition for some dry powder inhaler is related to flow. Radiolabelled budesonide inhaled from a Turbuhaler® by 10 healthy volunteers revealed that the mean (SD) total lung dose was 14.8% (3.3) of nominal dose when using a slow inhalation flow (36 L min^{-1}) and 27.7% (9.5) for a fast flow (58 L min^{-1}). Hence the inhalation of budesonide at the fast flow resulted in a significant increase in lung deposition compared with the slower flow which was matched by a decrease in deposition in the oropharynx and mouthpiece (Borgstrom et al. 1994).

A study has shown that when 10 asthmatic patients inhaled terbutaline from a Turbuhaler® at slow (28 L min^{-1}) and fast (60 L min^{-1}) rates, significantly more drug was deposited in the lung using the fast inhalation flow than with the slow flow. The data showed that the inhalation of terbutaline sulphate via the Turbuhaler® at the fast flow resulted in a significant increase in lung deposition ($p<0.01$), which was almost exactly matched by a significant decrease in deposition in the oropharynx ($p<0.01$), and the percentages of the dose left in the mouth piece and in the exhaled air were similar for the two inhalations (Newman et al. 1991). These studies highlight that the flow dependent effects are due to the device and the formulation rather than the drug mirror data reported by in-vitro studies (Ross and Schultz, 1996).

Gamma scintigraphy was used to measure the in-vivo deposition of salbutamol in 10 healthy volunteers. Measurements were made using a fast (46 L min-1) and a slow (28.3 L min^{-1}) inhalation (Pitcairn et al, 1994). The data showed that the percentage of the dose (SD) deposited in the lungs using the fast inhalation flow was 14.1 I (3.2) %) which was significantly greater than the dose deposited when the slower inhaled flow was used (11.7 (2.3) %). Deposition of labelled salbutamol in the central, intermediate and peripheral regions of the lung was higher at the fast flow rate than at the slow flow rate, but the ratio between peripheral and central deposition was similar for the fast and slow rates, and the

majority of the dose was deposited in the oropharynx (means 80.3 and 78.7% at fast and slow flow rate respectively).

Lung deposition of 20mg sodium cromoglycate powder inhaled via the Spinhaler has been measured in 10 healthy volunteers at fast (120 L min^{-1}) and slow (60 L min^{-1}) inhalation flow. Optimum lung deposition was achieved when powder was inhaled at 120 L min-^{1} (mean 17.1% of the dose) compared to the slow inhalation flow 60 L min^{-1} (mean 5.5%) (Newman et al, 1994), similar results were reported by Vidgren et al., (1988) where the mean lung deposition of labelled sodium cromoglycate inhaled via the Rotahaler at a slow inhalation flow rate (60 L min^{-1}) was 6.2%.

A further study, reported by Pitcairn et al (1997), also showed flow dependent lung deposition when radiolabelled nedocromil was inhaled by healthy volunteers using an Ultrahaler at a slow (42 L min^{-1}) and fast (75 L min^{-1}) inspiration flow. The total lung dose was higher for the fast rate, a finding which is consistent with the above reports for other devices. The mean total lung deposition of nedocromil at the slow and fast flow was 9.8% and 13.3% respectively (Pitcairn et al. 1997).

Studies with the Novolizer® have shown that a comparable or better lung deposition can be achieved compared with the Turbuhaler at similar or higher inspiratory flows. Newman et al. (2000) evaluated the lung deposition of budesonide (200 µg single radiolabelled dose) delivered through the Novolizer® at different targeted flows (45, 60 and 90 L min^{-1}) and compared this with the lung deposition of budesonide delivered through the Turbuhaler at a high flow (60 L min^{-1}), using a gamma scintigraphy technique. The study included 13 healthy volunteers and had a randomized cross-over design. Results showed that the median percentage deposition of budesonide in the lungs achieved through the Novolizer was 32.1 %, 25% and 19.9 % for measured flows of 99, 65 and 54 L min^{-1} respectively. At the lowest flow of 54 L min^{-1}, comparable to that for the Turbuhaler 58 L min^{-1}, a similar percentage of the drug was delivered to the lung. However, at higher flows, the

Novolizer® delivered significantly more drug to the lungs and resulted in significantly less deposition in the mouth and oropharynx compared to the Turbuhaler. The pattern of regional lung deposition in the different regions of the lungs achieved through the Novolizer® showed good penetration of the lung periphery independent of the flow rates tested as shown in Table 2.8.

Table 2.8 Mean percentage deposition of budesonide inhaled via the Novolizer® and Turbuhale®r at different flows (adapted from Newman et al., 2000)

	Novolizer®			Turbuhaler®
Targeted flow ($Lmin^{-1}$)	90	60	45	60
Measured flow ($Lmin^{-1}$)	99	65	54	58
Mouthpiece %	9.5	15.6	17.3	11.6
Exhalation filter %	0.8	0.6	0.2	0.2
Oropharynx %	57	61.6	60.9	71.9
Lungs %	32.1	25	19.9	21.4
Central lung%	10.6	7.8	6.3	5.9
Intermediate lung %	10.9	8.9	6.7	7.3
Peripheral lung %	8.5	7.8	6.5	4.8
Peripheral/central ratio	0.9	1	1	0.9

Although lung deposition with the Novolizer® increased with inhalation flow the penetration index (peripheral: conducting airway ratio) was the same.

(b) In-vitro flow dependent dose emission

Total dose and fine particle dose emission together with the determination of the aerodynamic particle size distribution provide useful data to highlight the in-vivo results described above. Palander et al. (2000) demonstrated the property of flow dependent dose emission from dry powder devices. Figure 2.18 shows that for some dry powder devices such as the Accuhaler the effect of the inhalation flow is low whilst it is greater for the Turbuhaler.

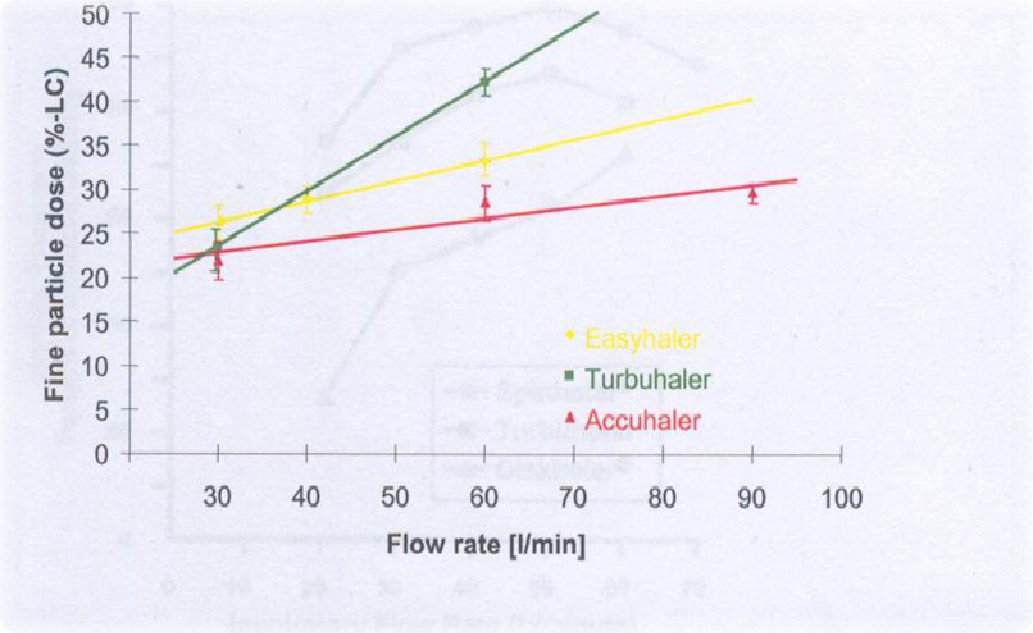


Figure 2.18 Fine particle dose (percentage of label claim) determined for three salbutamol containing multidose dry powder inhalers at inhalation flows (30-60 L/min) (adapted from Palander et al., 2000**).**

The emitted dose from three different DPIs (Spinhaler, Turbuhaler and Diskhaler) at different inhalation flows also showed significant sensitivity of dose delivery (Figure 2.19). The Diskhaler was the most sensitive to inhalation flow, emitting <30% of the claimed label dose at 20 L/min (de Boer et al, 1996).

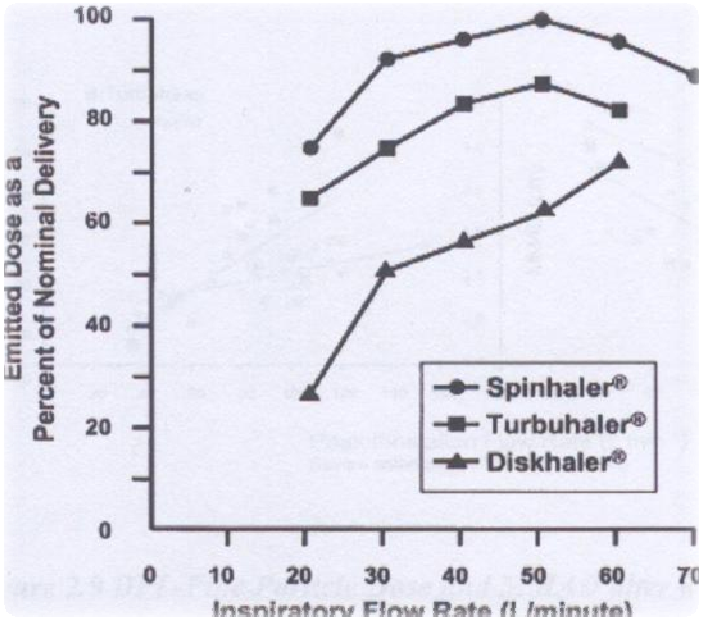


Figure 2.19 Emitted doses of three DPI devices at different inhalation flows (adapted from de Boer et al, 1996).

Furthermore, inhalation volume (which translates inspiration time) is of importance in parallel to inspiratory flow, when considering dose emission from a dry powder inhaler.

The effect of inspiration time (which translates to inhalation volume) on dose emission from the dose systems of Diskhaler® (blister), Turbuhaler® (reservoir) and Spinhaler® (hard gelatine capsule) over inhalation flow range (20-60Lmin^{-1}) was studied by de Boer, et al, (1996). In that study it was found that the effect of inspiration time on the dose emission from the Turbuhaler® and the Diskhaler® over the same flow range was negligible for all four inspiration times (0.5, 1.5, 3.0 and 6 s) used. On the other hand, the effect of inspiration time was evident for the Spinhaler®. After 0.5s of inspiration time, only a fraction of the dose was discharged from the capsule compared to the longer time, thus the DPI showed dependence on both inspiration flow and inspiration time (inhaled volume). De Boer and colleagues attributed the difference in behaviour to different designs and the type of powder formulation (de Boer et al., 1996). For the Spinhaler®, dose emission depends on the flow properties of the drug formulation and the rotation speed of the capsule such that the capsule is emptied. They further reported that inhalation flows <

50-60 $Lmin^{-1}$ seemed to be insufficient for complete emptying of the capsule, even at longer inspiration times of 3-6 s. A study by Hawskworth, et al. (2000) has shown that inhalation volume for asthmatic patients when they inhaled through a DPI was about 2L (which translates to inspiration time of 2s, if 60 $Lmin^{-1}$ is used).This means that an asthmatic patient will require longer time to empty a single-dose capsule and this may be achieved using two inhalations per dose. Hence for this type of DPI inhaling twice using the same capsule has been recommended in order to allow emptying of the capsule as shown in Figure 2.20.

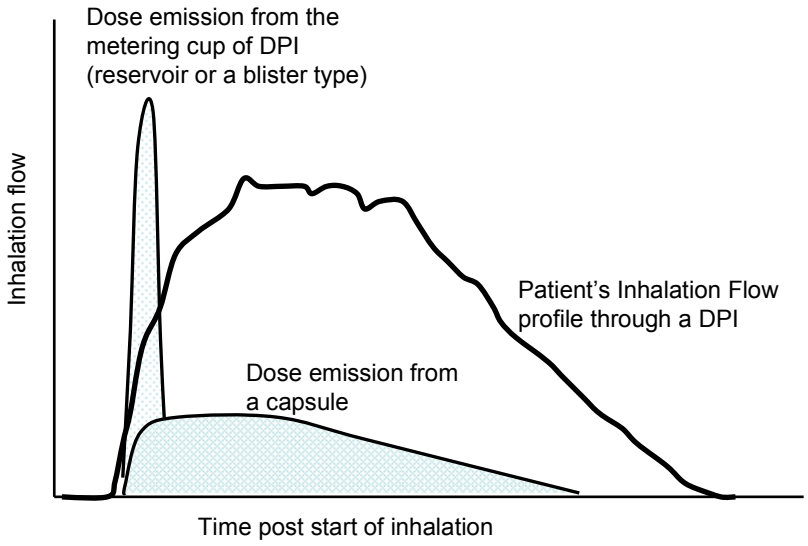


Figure 2.20 The relationship between a patients inhalation flow profile through a DPI and dose emission from two types of DPI (adapted from Chrystyn,unpublished)

A study using the electronic lung (ex-vivo) method confirmed that the fine particle dose increased with inhalation flows through the Accuhaler and the Turbuhaler devices, hence the increase in lung deposition with flow (Tarsin et al. 2006). They also reported that the MMAD decrease as the inhalation flow increased (Figure 2.21). The smaller MMAD values would counteract the increased potential for more central deposition in the lungs with fast inhalation flow. Thus a higher fine particle dose and smaller MMAD explain why

the lung deposition for the Novolizer® increased with the flow but the penetration index did not change (Newman et al. 2000).

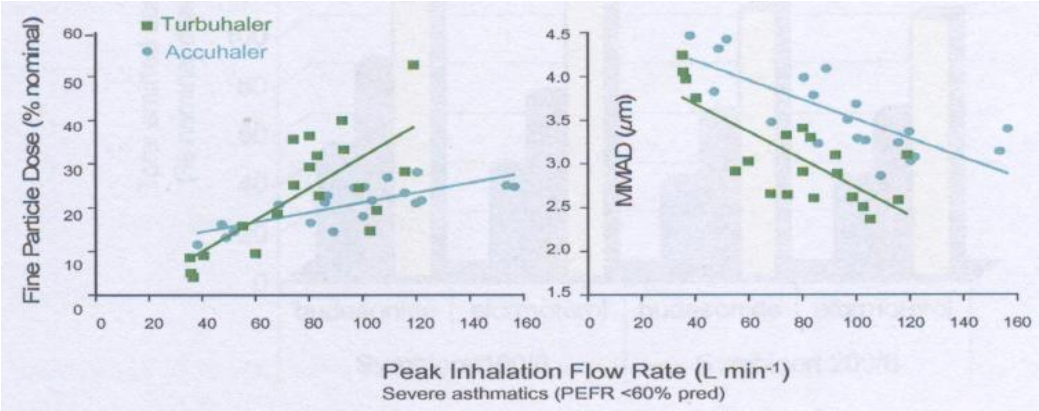


Figure 2.21 DPI-Fine particle dose and MMAD alter with inhalation flow (adapted from Tarsin et al, 2006).

Tarsin and colleagues also measured the in-vitro dosage emission and the fine particle dose (FPD) from 100/6 and 200/6 Symbicort Turbuhaler® (budesonide and eformoterol) at different flows (30, 60 and 90 $Lmin^{-1}$). The results are as shown in Figure 2.22. The data show that the amounts of budesonide and eformoterol emitted from the Symbicort 100/6® and Symbicort 200/6® inhalers were affected by the increased inhalation flow (Tarsin et al. 2004). Also the results demonstrate that the average total dose emissions at 90 L min-1 were all greater than 100%. Each determination at 90 $Lmin^{-1}$ was carried out after doses had been discharged using a flow rate of 60 $Lmin^{-1}$. The data obtained suggest that at 60 $Lmin^{-1}$ the total dose emission is not 100%. Thus total dose emission of >I00% at 90 L min^{-1} suggests that at the higher flow, some residual dose from the previous inhalation may also be inhaled. This study also showed that from the Turbuhaler ® there was an intra-inhaler variability of the dose emitted from the same inhaler and that there was also an inter-device variability.

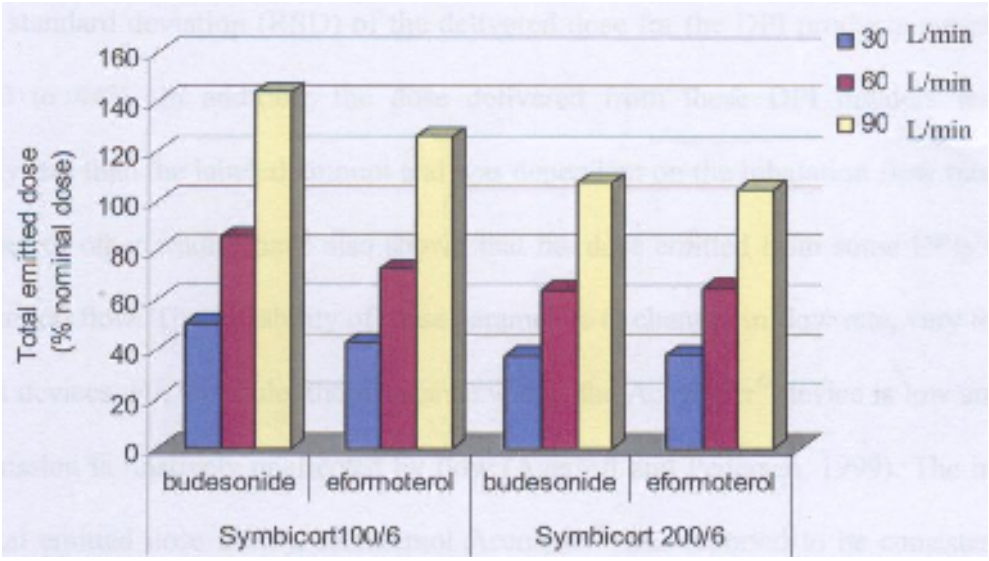


Figure 2.22 Mean (% nominal dose) emitted from the Symbicort100/6® and the Symbicort 200/6 Turbuhalers® for budesonide and eformoterol at different flows (adapted from Tarsin et al, 2004)

Ross and Schultz (1996) compared the emitted dose delivery from a Diskhaler (Ventodisk 200µg), Rotahaler® (Becotide l00µg), and Turbuhaler® (Pulmicort 200µg) at two different flows (30 and 60 $Lmin^{-1}$). Regardless of the flow, the amount of drug delivered from the DPI inhalers was variable from device to device. The relative standard deviation (RSD) of the delivered dose for the DPI products ranged from 10 to 44%. In addition, the dose delivered from these DPI inhalers was generally less than the labelled amount and was dependent on the inhalation flow. A number of other studies have also shown that the dose emitted from some DPIs is dependent on flow. The variability of these parameters to changes in flow varies for different devices. For example, the resistance within the Accuhaler® device is low and dose emission is relatively unaffected by flow (Agertoft and Pedersen, 1999). The in- vitro total emitted dose from a salbutamol Accuhaler® was reported to be consistent throughout the life of the device with very similar results obtained at flow of 30- 90 $Lmin^{-1}$ (Malton et al, 1996). In another study, (Gunawardena et al. 1995)

have shown that young patients (three years old) can achieve the desired flow of 30 L min^{-1} and above when using an Accuhaler®, at which they would received 100% of the labelled dose. The Turbuhaler® dose emission has been shown to be more dependent upon flow than the Accuhaler®. The Turbuhaler® showed greater variation of dose throughout its life (Malton et al, 1995). These researchers found that the fine particle fraction of terbutaline from a Turbuhaler was just 8.3% of the label claim at a flow of 28.3 L min^{-1}. However, at a flow rate of 60 L min^{-1} this was increased to 25.8% of the label claim.

In - vitro (Barrowcliffe et al. 1997) and clinical, (Newhouse et al. 1999) studies using Clickhaler® together with lung deposition using gamma scintigraphy (Warren and Taylor 1998) and urinary pharmacokinetics methods (Chege and Chrystyn 2000) have shown that inhalation flow is a minor issue. Asthmatic patients aged 6 or more could produce sufficient inspiratory airflow to operate the Clickhaler® and receive the required therapeutic outcome (Newhouse et al., 1999 b).

The results of these studies indicate there is a difference in the emitted dose (fine particle dose) from dry powder inhalers with respect to the inhalation flows used. This variation of the emitted dose with respect to the inhalation flow differs from device to device.

(c) How patients use inhalers

Different ways of performing the inhalation manoeuvre affect lung deposition. All currently available devices are flow-dependent; MDIs should be used with a flow as low as possible (Newman., 1982; Newman et al, 1995) and DPIs will deliver a larger pulmonary dose at a high inhalation flow than at a low flow (Newman et al, 1991 ; Pitcairn et al., 1994; Borgstrom, 1994). Hence the patient information leaflets for MDIs recommend an inhalation that is slow, deep and prolonged, whilst for DPIs the inhalation manoeuvre should be fast from the start of the inhalation and sustained for as long as possible.

To ensure effective drug delivery to the lung, the turbulent airstream created in any DPI during inhalation must be sufficient to produce an adequate aerosol cloud with respirable

fine particles. This involves a balance between the design of the DPI, the formulation and the patient's generated inhalation flow. A period of breath holding after inhalation improves delivery of inhaled medication because it gives time for the process of sedimentation.

A meta-analysis of inhaler studies concluded that maximum inhaler scores were achieved by 59% of those using a DPls. 43% with the MDI alone and 55% with the MDI when it was attached to a holding chamber (Brocklebank et al. 2001). These data support the intuitive view that DPls are used correctly more often than MDls alone. However patient instruction in correct inhaler use eliminated that difference, increasing the percentage of subjects who showed correct technique to 65% for DPls and 63% for MDls (Wright et al. 2002). Such data provide evidence of the positive effect of teaching patients to achieve correct inhaler use. Thus an increase in the lung deposited fraction would have a valuable response effect. Lung deposition and, therefore, clinical response to inhaled medication depends on the inhalation technique of the patients. The degree of lung deposition increases as a patient uses their inhaler correctly. A recent study by (Leach et al. 2005) showed that patients received optimal lung deposition with a good technique compared to patients with a poor technique.

Many asthmatic and COPD patients have been found to be unable to use DPls and MDI adequately. (Melani et al. 2004) found that similar percentages of patients failed to perform essential steps needed for reliable lung deposition of inhaled aerosol from a MDI three different (Aerolizer®, Turbuhaler® and Diskhaler®). Figure 2.23 shows the percentages of patients of patients who failed to correctly perform steps in inhaler use that are critical for a reliable drug delivery to the lungs with three different dry powder inhalers and a metered dose inhaler.

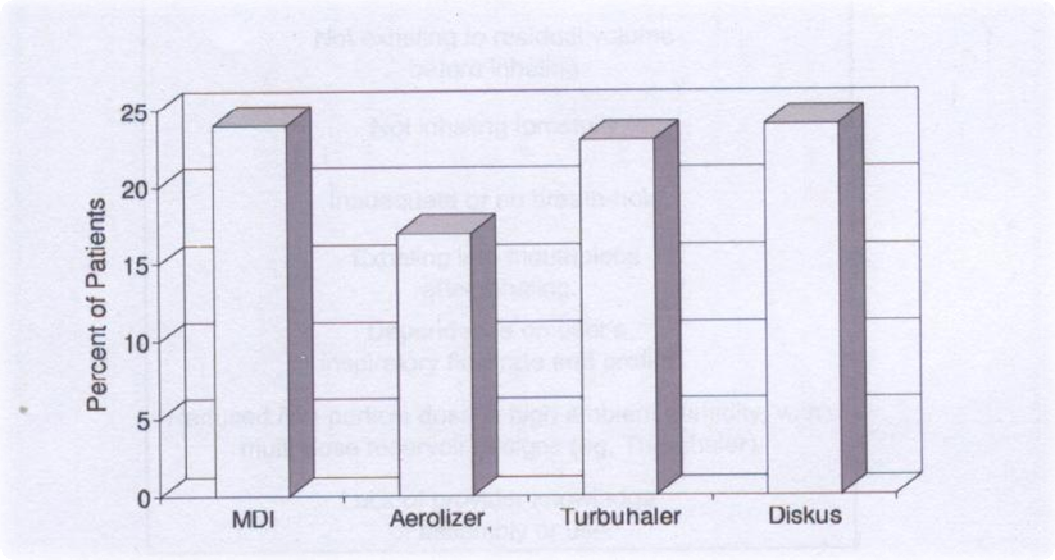


Figure 2.23 Failure to correctly perform essential steps through different inhalers by patients (adapted from Melani et al, 2004).

A similar study assessed inhalation technique among asthmatic and COPD patients and found that 52% of patients exhibited mistakes with the Diskhahler® 73% the Rotahaler® and 80% the Ingelheim DPIs (Van Beerendonk et al. 1998). In one study of patients with COPD 58% reported no problems using Accuhaler compared with only 11% using the Handihaler®; 72% of the patients also reported that they would not be happy to use the Handihaler® (Moore and Stone 2004).

Studies suggested that 28-68% of patients with asthma have problems using their MDI or DPI sufficiently well to benefit from the dose (Raul 2006). Table 2.9 summarises the range of problems patients have with the use of these inhalers (Crompton 1982; Rau 2005; Rönmark et al. 2005; Rau 2006).

Table 2.9 Errors in patients' use of MDIs and DPIs (adapted from Chrystyn, unpublished)

Error	per cent of patients	
	MDI	DPI
Failure to co-ordinate actuation and inhalation	27	-
Inadequate or no breath hold after inhalation	26	23
Too rapid inspiration / not inhaling forcibly	19	17
Inadequate shaking / mixing before use	13	-
Cold Freon effect	6	-
Actuation at total lung capacity / Not exhaling to residual volume before inhaling	4	24
Multiple actuations during single inspiration	3	-
Inhaling through nose during actuation	2	-
Exhaling during activation / through the mouth piece	1	19
Putting wrong end of inhaler in mouth	<1	-
Holding device in wrong position / incorrectly	<1	35
Exhaling into the mouth piece after inhalation	-	20

In general, a review by Crompton et al, (2000) found that approximately 50% of the patients in Europe are unable to use their inhaler devices correctly.

Also, children generally cannot use DPIs properly. Pedersen (1990) reported that young children did not have enough inspiratory effort to use the Turbuhaler®. (de Boeck et al. 1999) showed that although the vast majority of children older than 8 years could perform every step of the inhalation manoeuvre through the Turbuhaler correctly after training, only half of the children younger than 8 years were able to do so. Thus, some studies have recommended that the Turbuhaler device was not suitable for pre-school children because they cannot use the inhaler correctly (Pedersen et al, 1990; Bisgaard et al, 1994). Table 2.10 shows the critical steps for using various inhalers (Fink and Rubin 2005).

Ideally GPs and nurses should teach patients how to use their inhalers correctly when they are prescribed. Alternatively the pharmacist could play an important role in teaching

patients correct inhalation technique when the drug is prescribed for the first time and also when the prescription is repeated. A number of methods have been used to teach the patients a correct inhalation technique these methods include: patient information leaflets, face to face demonstration and multimedia methods such as video demonstrations and touch screen computer methods. A recent study showed that the use of multimedia and touch screen computer improved the patient's inhalation technique more effectively than provision of a patient information leaflet (Savage and Goodyer 2003). This form of education was acceptable to patients of all age groups.

Overall, the issue of correct use of inhalers is of critical importance in maintaining optimal asthma control as patients who misuse their inhalers tend to have less stable asthma than those who use their device correctly (Giraud and Roache 2002).

Table 2.10 Critical steps for using various inhalers (adapted for Finks et al., 2005)

pMDI	Rotahaler	Diskhaler	Diskus	Turbuhaler	Handihaler
1. Take cap off mouthpiece 2. Warm to room or hand temperature 3. Shake thoroughly 4. Exhale fully 5. Place mouthpiece between lips or 2 finger-widths in front of open mouth 6. Actuate as you begin a slow deep breath 7. Breath-hold 8. Place cap on mouthpiece	1. Insert capsule 2. Twist device to break capsule 3. Keep device level while rapidly inhaling dose 4. Breath-hold 5. Remove device from mouth and exhale away from device 6. Store device in a cool, dry place	1. Remove mouthpiece cover 2. Pull tray out from device 3. Place disk on wheel (numbers up) 4. Rotate disk by sliding tray out and in 5. Lift back of lid until fully upright so that needle pierces both sides of blister 6. Keep device level while rapidly inhaling dose 7. Breath-hold 8. Remove device from mouth and exhale away from device 9. Brush off any powder remaining within device once every week 10. Store device in a cool, dry place	1. Open the device 2. Slide the lever 3. Keep device level while inhaling dose 4. Exhale away from device, to residual volume 5. Inhale rapidly and fully 6. Breath-hold 7. Remove device from mouth and exhale away from device 8. Store device in a cool, dry place	1. Twist and remove cover 2. Hold inhaler upright (mouthpiece up) 3. Turn grip right, then left, until it clicks 4. Exhale away from device to residual volume 5. Inhale rapidly and fully. Inhaler may be held upright or horizontal for this step 6. Breath-hold 7. Remove device from mouth and exhale away from device 8. Replace cover and twist to close 9. Store device in a cool, dry place	1. Remove mouthpiece cover 2. Take capsule from package 3. Place capsule into the inhaler 4. Close the inhaler 5. Press button so that needle pierces both sides of capsule 6. Keep device level while inhaling dose rapidly and fully 7. Breath-hold 8. Remove device from mouth and exhale away from device 9. Brush off any powder remaining within device once every week 10. Store device in a cool, dry place

2.5.2.3 Characteristics of the patients

Each patient's ability to use inhaler devices correctly is pertinent to aerosol therapy. Many patients experience problems using their devices correctly. MDIs require co-ordination between the inhalation manoeuvre and the release of the dose from the device, which many patients; especially children and the elderly find difficult (Crompton 1982). In addition some patients naturally stop inhaling partway through inhalation because of their response to the cold aerosol hitting the back of their mouth (Fink, 2000).

Single dose (capsule) dry powder inhalers require each dose to be loaded separately into the device. This procedure requires manual skill that is difficult for the elderly, arthritic and disabled patients (Crompton 1990).

Dry powder inhalers (DPIs) are dependent on the patient's initial inspiratory effort to de-aggregate the powder formulation and generate drug particles in the optimal size range to reach the target sites in the lungs. Studies have shown that young children with asthma (Pedersen et al.; 1990) and patients with chronic obstructive pulmonary disease (COPD) (Nsour et al; 2000) have problems achieving the minimum required inhalation rate through a DPI. A reduced inspiratory flow can result in poor drug release and low pulmonary deposition (Newman et al. 1991; Pitcaim et al., 1994; Borgstrom 1994).

The state of the patient's airways may alter due to the disease progression. For example, inflammation , obstruction or mucus produces narrowing of the airways, which in turn will increase the linear velocity of air in those airways and so enhances impaction and produces a more central deposition pattern (Clarke 1990).

These studies highlight that although the patients are trained on how to use an inhaler device, if they do not have the ability to achieve the required inhalation manoeuvre through the device then dosing will not be as expected and could even be zero (Chrystyn, 2006).

It is, therefore, important that healthcare professionals should consider patients preference together with their competence to use an inhaler before prescribing. Carefully choosing the

most appropriate inhaler device along with teaching the patient correct inhalation technique can maximise asthma management and optimise overall clinical outcomes. Also consideration should be given to those inhalers that are least affected by the inhalation method used by the patient and those with more efficient lung deposition with reduced oropharyngeal impaction. Hence, the use of the In-Check Dial® by the healthcare professionals to identify a patient's inhalation rate through an inhaler and thus, the device to suit the patient's natural technique prior to prescribing is very important. Also the healthcare professionals should consider these issues before changing the dose of inhaled steroid or adding other treatments to the regimen of patients poorly controlled at any particular step of the guidelines.

2.6 The In-check Dial® (Clement Clarke International)

Each type of dry powder inhaler (DPI) is designed to emit a consistent fine particle dose over a range of inhalation rates. Thus it is important that the patient can achieve these rates when they use their DPI. The In-Check Dial® (Figure 2.24) has been introduced to identify a patient's inhalation rate through an inhaler and therefore the device to suit a patient's natural technique. The Dial is similar in appearance to a peak flow meter except that the patient inhales through it rather than a forced expiration. The mouthpiece has a dial that it can be turned so that it is set to simulate the resistance of several commonly used inhaled products such as Accuhaler®(Diskus), Clickhaler®, Easyhaler®, Easi-Breathe®, Autohaler®, and Turbuhaler®. This is achieved by altering the diameter of the inhalation orifice/hole such that the resistance is the same as that of the selected inhaler. The In-Check Dial® is designed to mimic the use of a specific inhalation device. The rate of inhalation is measured by reading the value on the meter. When the patient inhales through the In-Check Dial® the reading identifies the inhalation rate that would be obtained when using the inhaler for which the meter has been set for. The use of the Dial in the clinic should enable the prescriber to choose the inhaled device that requires minimum training

of the recommended inhalation technique. The In-Check Dial® has been externally tested in vitro by AEA Technology and found to provide similar resistance to the inhalation devices available (http://www.clement.clarke.com/inspiratory/In-check).

There has been no simple way to determine if the patient can use a preferred inhaler properly or not. The literature highlights that there is a link between the emitted dose (particularly the fine particle dose), total lung deposition and ultimately clinical response (Newman et al. 1991; Engel et al. 1992). The results of these studies indicate there is a difference in the emitted dose from dry powder inhalers with respect to the inhalation rates used. Studies have highlighted the potential of the In-Check Dial® to identify inhalation rate and thus inspiratory effort of all type of patients using different dry powder inhalers (Tarsin et al. 2000). In these studies the In-Check Dial® showed that 6 (8%) out of 74 COPD patients (Nsour et al. 2001) together with 48 (Emeryk et al. 2000; Amirav et al. 2005) children out of 64 (75%) and 57 (30%) respectively could generate an inspiratory flow of >60 $Lmin^{-1}$ when using the Turbuhaler. Also a study by (Tarsin 2000) showed that the measurements of inhalation rates using the Dial to be as accurate as the electronic measurements (using an Inhalation Profile Recorder) when patient inhaled through the Accuhaler® and Turbuhaler® respectively. In the clinic the In-Check Dial® can be used to identify an inhaler that patient can use without training.

Figure 2.24 In-Check Dial® (Clement Clarke International Limited, UK (**http://www.clement.clarke.com/inspiratory/In-check)**)

2.7 Methods of determination of the bioequivalence of inhaled products

In the Pharmacopoea two products may be pharmaceutically equivalent if they have the same composition and in-vitro performance. They may be bioequivalent if they have the same pharmacokinetic and lung deposition profiles. They may be clinically equivalent if they have the same therapeutic effects and side effects (Barry and O'Callaghan 2003).

There are four methods available for determining the bioequivalence of different inhaled products: simulated in vitro dose emission and particle size distribution measurements, in vivo gamma scintigraphy (radioaerosol drug deposition), pharmacokinetic, and pharmacodynamic. In combination, these methods can indicate the fate of an inhaled drug as the pulmonary fate of the aerosolized drug is influenced by where the aerosol particle is deposited in the lung (Chrystyn 2000; Chrystyn 2001; Mobley and Hochhaus 2001).

2.7.1 In-vitro methods

In vitro methods are a major component of quality assurance procedure used to determine the pharmaceutical performance of the inhaled products using parameters such as the total emitted dose, uniformity of dose, aerodynamic particle size distribution and the fine particle dose. The efficacy and safety of an inhaled product are dependent on these parameters. In addition, they are often used to predict lung deposition. The most used techniques are inertial separation methods and laser diffraction. Microscopic methods have also been used.

Aerosol particles are not perfectly spherical, instead they are of an uneven shape, weight and surface area that would be impossible to be described accurately by any one variable. Instead, such particles are generally described by their aerodynamic diameter. This is the diameter of a unit density sphere that has the same settling velocity in air as the particle. This aerodynamic diameter takes into account particle density, shape and size (Hickey 1992). The mass median aerodynamic diameter (MMAD) of an aerosol is the diameter that separates the mass of the particles equally by 50%.The MMAD can be determined by

plotting the cumulative mass of active ingredient versus the cut-off diameter on a log probability graph (BP, 2001). The MMAD can then be determined by reading from the (sigmoid) curve at the 50% co-ordinate. The GSD is a measure of the polydispersity, or spread, of an aerosol. A monodispersed aerosol has a GSD of 1 and heterodispersed aerosol has a GSD greater than 1.2. The amount of an aerosol which contained particles with an aerodynamic diameter less than 5μm, is generally referred to as the fine particle dose [FPD] (Newman 1991). This is the quantity of drug in the prescribed dose of an inhaled product that is generally considered to be of a size capable of penetrating the lung during an inhalation i.e. respirable amount. Since the label claims of the various inhalers differ, for ease of comparison in terms of performance, the FPD is expressed as a percentage of the nominal (label claim) dose to give the fine particle fraction (FPF). Although the respirable fraction does not equal the amount of aerosol deposition in the lung, it provides an estimate of the fraction of the dose that has the potential to be deposited into the lungs (Dhand and Fink 1999; Barry and O'Callaghan 2003).

2.7.1.1 DPI dose emission unit:

The DPI dose emission unit, as shown in Figure 2.25, is used to perform those tests specified by the Pharmacopoeia (European Pharmacopeia 2007; British Pharmacopoeia 2008; United States Pharmacopeia 2009) that relate to content uniformity namely 'Delivered Dose Uniformity' and in the case of a multi-dose DPI, 'Dose Uniformity over the Entire Contents'. In the case of DPIs, both the emitted and fine particle dose is affected by the strength and duration of the patient's inspiration (Newman et al. 1991; Ross and Schultz 1996). Furthermore, different inhalers provide varying degrees of resistance to flow (Clark and Hollingworth 1993). For these reasons, according to the compendial methods, it is essential, particularly when testing DPIs of intermediate to high flow resistance, to determine the appropriate test flow and duration based on the pressure drop developed over the specific inhaler under test. Assuming this is done, it is then important

to ensure that critical (sonic) flow occurs in the flow regulating valve employed in the system. This ensures that the flow through the dose emission unit is set as required and that it is unaffected by minor fluctuations in the pump. The resulting airflow that produces a drop of 4.0 kPa over the inhaler to be tested, should then be used for the determination of the delivered dose and particle size distributions as recommended by the compendial methods (European Pharmacopeia 2007; British Pharmacopoeia 2008; United States Pharmacopeia 2009).

The only exception to this criterion is for those low resistances DPI that produce a flow in excess of 100 L min^{-1}. In this case, a flow of 100 L min^{-1} should be used.

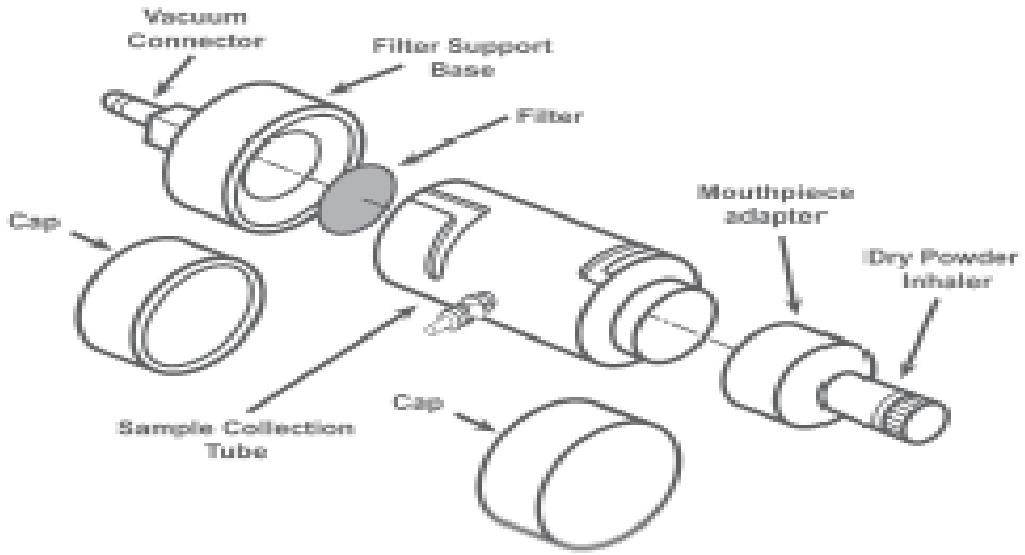


Figure 2.25 Parts and fitting of the DPI dose emission unit (Reproduced from Copley 2008)

2.7.1.2 Characterisation of the emitted dose:

Inertial impaction as a size separation factor by cascade impaction methods has been widely used as the 'gold standard' to determine the aerodynamic characteristics of the emitted dose from aerosols (European Pharmacopeia 2007; British Pharmacopoeia 2008; United States Pharmacopeia 2009). The application of this class of particle size analysis to the assessment of medical aerosols has recently been extensively reviewed, focusing on the

types of impactor that are in current use together with their strengths and limitations for measurements with the different classes of inhalers (Mitchell and Nagel 2003).

(a) Twin Stage Impinger:

The Twin Stage Impinger shown in Figure 2.26 (United States Pharmacopeia 2005) can be operated using inhalation flows of 30 and 90 L min^{-1}. It has been retained in the Pharmacopoeias because of its value as a simple and inexpensive quality control tool (Newman and Kenyon 1994).

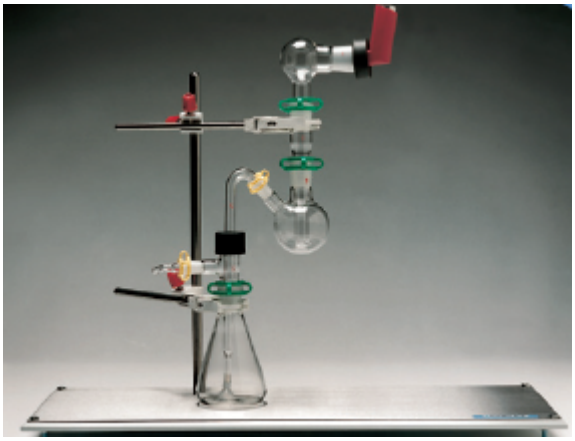


Figure 2.26 The Twin Stage Impinger (Reproduced from Copley, 2008).

(b) Multistage Liquid Impinger:

The Multistage Liquid Impinger [MSLI] (United States Pharmacopeia 2005) consists of a metal throat, impaction stages and a final filter. The MSLI operates at 60 L min^{-1} and has cut-off diameter of 25, 13, 6.8, 3.1 and 1.7 μm, thus giving a much more detailed particle size distribution then the Twin Stage Impactor (Figure 2.27). However the number of cut-off diameters is limited.

Figure 2.27 The Multi-stage Liquid Impinger (Reproduced from Copley, 2008)

(c) Anderson Cascade Impactor:

As shown in Figure 2.28 the Anderson Cascade Impactor (ACI) consists of a stack of eight plates, each containing a series of precision drilled holes, and a final filter stage (European Pharmacopeia 2001; British Pharmacopoeia 2005; United States Pharmacopeia 2005). The diameter of the holes decreases progressively in each succeeding stage. Therefore, the jet velocity increases as a particle travels through the impactor. The standard ACI is designed to operate at a flow of 28.3 L min^{-1} with stage cut-off diameters of 9, 5.8, 4.7, 3.3, 2.1, 1.1, 0.65 and 0.43 μm, respectively. This method allows a more detailed description of the particle size distribution than either the MSLI or the Twin Impinger. Although the ACI is designed to be used at inhalation flows of 28.3 L min^{-1}, modifications are available for the use at high flows 60 and 90 L min^{-1}. For an inhalation flow of 60 L min^{-1} stages 0 and 7 are removed and replaced by stages -1 and -0 on the top of the ACI. For an inhalation flow of 90 L min^{-1} stages 0, 6 and 7 are removed and replaced by stages -2, -1, and -0 on the top of the ACI. Alternatively the standard ACI can be operated at different flows with the cut-off stages altered according to the following equation ($ECD_{F2} = ECD_{28.3}\,(28.3/F2)^{0.5}$). Where ECD_{F2} = the Effective Cut-Off Diameters at the other flow; $ECD_{28.3}$ is the ECD at the manufacturers flow (28.3 L min^{-1}) and F2 is the other flow rate in L min^{-1} (Van Oort 1995). When sampling DPI aerosols, a pre-separator with a small amount of solvent is added to the ACI to prevent those particles greater than 10μm from bouncing into the ACI stages

(Figure 2.22). Furthermore, it is important in predicting lung deposition of inhaled products to use conditions that simulate normal patient's use. Thus, the need to use the Andersen Cascade Impactor containing modification kits together with the mixing inlet (Copley 2003) that allows the determination of dose emission and aerodynamic particle size distribution of dose from DPIs at a variety of inhalation rates and for different inhaled volumes.

.

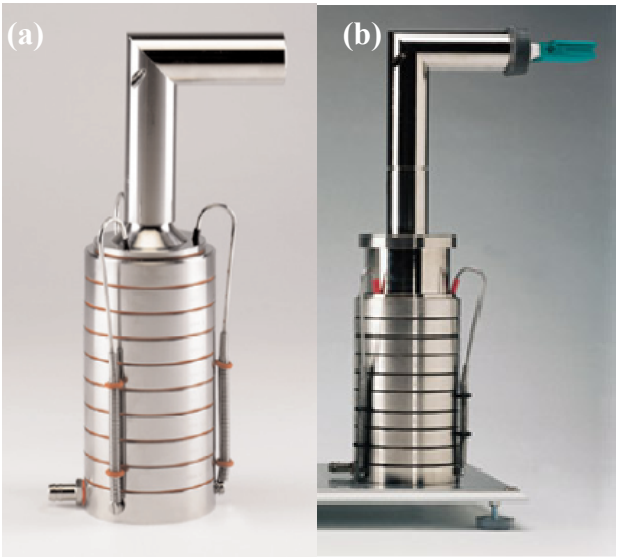


Figure 2.28 (a) The Anderson Cascade Impactor set for MDI. (b) The Anderson Cascade Impactor set for DPI (Reproduced from Copley, 2008).

(d) Next Generation Impactors:

The Next Generation Impactor has been designed specifically for pharmaceutical inhaler testing (Figure 2.29). This impactor has seven stages and is intended to operate at any inhalation flow between 30 and 100 L min^{-1}.

The cut-off size ranges from 0.54 µm to 11.7 µm aerodynamic diameter at 30 L min^{-1} and 0.24 µm to 6.12 µm at 100 L min^{-1}. The NGI has several features to enhance its utility for inhaler testing:

1. Particles deposited on collection cups are held in a tray from the impactor as a single unit, facilitating quick sample turn-around times if multiple trays are used.

2. The user can add up to approximately 40 ml of an appropriate solvent directly to the cups for more efficient drug recovery.

3. The Micro-orifice Collector (MOC) captures, in its collection cup, extremely small particles normally collected on the final filter of other impactors. The particles captured in the MOC cup can be analyzed in the same manner as the particles collected in the other impactor stage cups.

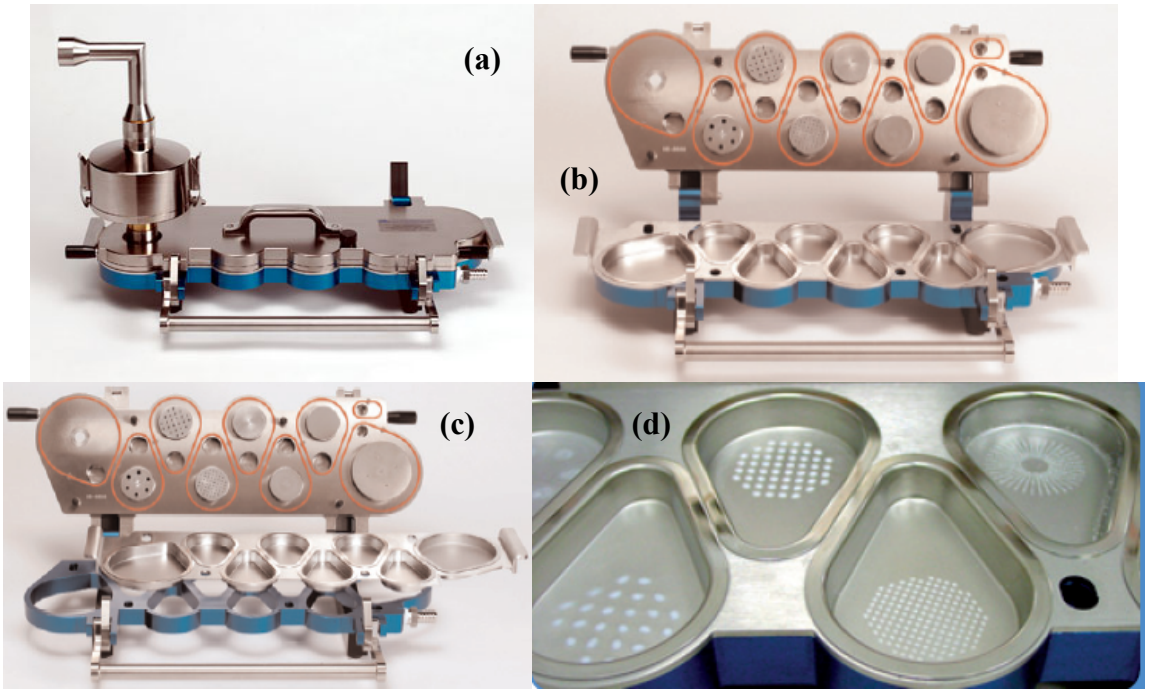


Figure 2.29 Next Generation Impactor (Reproduced from Copley, 2008). (a) NGI including preseparator and induction port. (b) NGI (open view) showing nozzles & collection cups. (c) NGI (open view) showing cup tray removed. (d) Collection cups showing typical deposition pattern.

2.7.1.3 Principles of operation of the cascade impactors

The term 'impactor' is generally used for an instrument where the particles 'impact' on a dry impaction plate or cup. If the collection surface is liquid, as in the case of the multi

stage liquid impinger, then the term ‘impinger’ is used. The general principles of inertial impaction apply to both ‘impactors’ and ‘impingers’

Cascade impactors operate on the principle of inertial impaction. Each stage of the impactor comprises a single or series of nozzles or jets, as shown in Figure 2.30, through which the sample laden air stream is drawn directing any airborne particles towards the surface of the collection plate for that particular stage. Whether a particular particle impacts on that stage is dependent on its aerodynamic particle size. Particles having sufficient inertia will impact on that particular stage collection plate, whilst smaller particles with insufficient inertia will remain entrained in the air stream and pass to the next stage where the process is repeated.

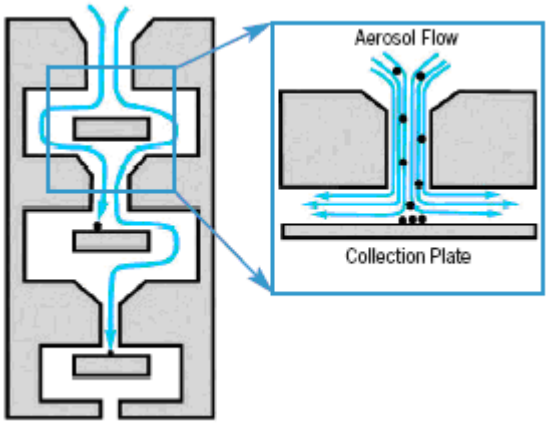


Figure 2.30 Principle of cascade impactor operation (Reproduced from Copley, 2008).

The stages are assembled in a stack, in order of decreasing particle size. As the jets get smaller, the air velocity increases and finer particles are collected. Any remaining particles are collected on a final filter. At the end of the test, the particle mass relating to each stage collection plate is recovered using a suitable solvent and then analysed usually using HPLC to determine the amount of active drug actually present.

By analysing the amount of drug deposited on the various stages in this manner, it is then possible to calculate the Fine Particle Dose (FPD) and Fine Particle Fraction (FPF) as well

as the Mass Median Aerodynamic Distribution (MMAD) and Geometric Standard Deviation (GSD) of the active drug particles collected.

In some instances, particles may bounce in response to impact when they contact the collection plate, in which case they are normally re-entrained into the air stream and carried to a lower stage. This can be a particular problem with a DPI and certain MDIs (where measurements are based on a limited number of actuations from the inhaler). This tendency may be avoided by coating the collection plate with a suitable surface coating (Allen 1990). Particle deposition on parts of the impactor other than the collection plates is called 'inter-stage losses' (Kamiya et al. 2004).

2.7.2 In vivo methods

2.7.2.1 Pharmacokinetic methods (using plasma or urine samples):

The Pharmacokinetic methods exploit the rapid absorption of drugs through the lung and the lag time for the drug to be absorbed following oral administration to determine the relative bioavailability of inhaled products. The dose emitted from an inhaler either enters the systemic circulation via the lungs or through being swallowed and absorbed in the gastrointestinal tract as shown in Figure 2.31. Also the gastrointestinal absorption of any drug that is swallowed can be blocked by the coadministration of oral charcoal thereby allowing the determination of lung deposition. Lung absorption (hence effective dose lung dose) can also be distinguished by the timing of sample collection (Hindle and Chrystyn, 1992; Lipworth 1996).

Both drug plasma concentration (Lipworth and Clark, 1997) and urinary excretion data (Hindle and Chrystyn, 1992; Chege et al, 1997) have been used to assess salbutamol lung deposition and relative bioavailability of inhaled products. Plasma studies can be difficult to perform due to high volume of distribution of salbutamol (being polar and basic)

(Morgan et al, 1998) resulting in very low plasma concentration after an inhalation (Chrystyn, 1994).

To compare lung deposition of inhaled products, Lipworth and Clark (1997) performed a plasma pharmacokinetic study in which a breath activated MDI and two DPIs (Diskhaler and Accuhaler) delivered 200µg salbutamol to 10 healthy volunteers over 6 minutes. Plasma concentrations were measured at 5, 10, 15 and 20 minutes post inhalation. Maximum concentration values reported were: 4.35 ng/ml (Diskhaler), 3.98ng/ml (MDI) and 3.22ng/ml (Accuhaler). Both the Diskhaler and the MDI produced significantly ($p<0.05$) higher plasma concentration than the Accuhaler.

The urinary pharmacokinetic has been introduced to overcome the problem associated with low plasma concentrations. Due to the relatively small volume of urine with respect to systemic volumes of distribution, the drug concentrations in urine are much high than those measured in the plasma (Chrystyn, 1994). By collecting urine up to 24 hours post dose, via inhalation and oral administration, it possible to determine the relative bioavailability of salbutamol to the body (Hindle and Chrystyn, 1992). However, to identify lung deposition, methods that distinguish between absorption via the lung and the swallowed portion are necessary.

The use of oral charcoal doses taken before and after the inhalation to block the gastrointestinal absorption of the oral ingested portion has been demonstrated (Borgstrom and Nilsson 1990). However, because this method uses oral charcoal it would be unethical to extend the method to patient studies due to their concomitant oral therapy (Chrystyn 2000). Lung absorption can also be distinguished by timing of urine sample collections (Hindle and Chrystyn 1992).

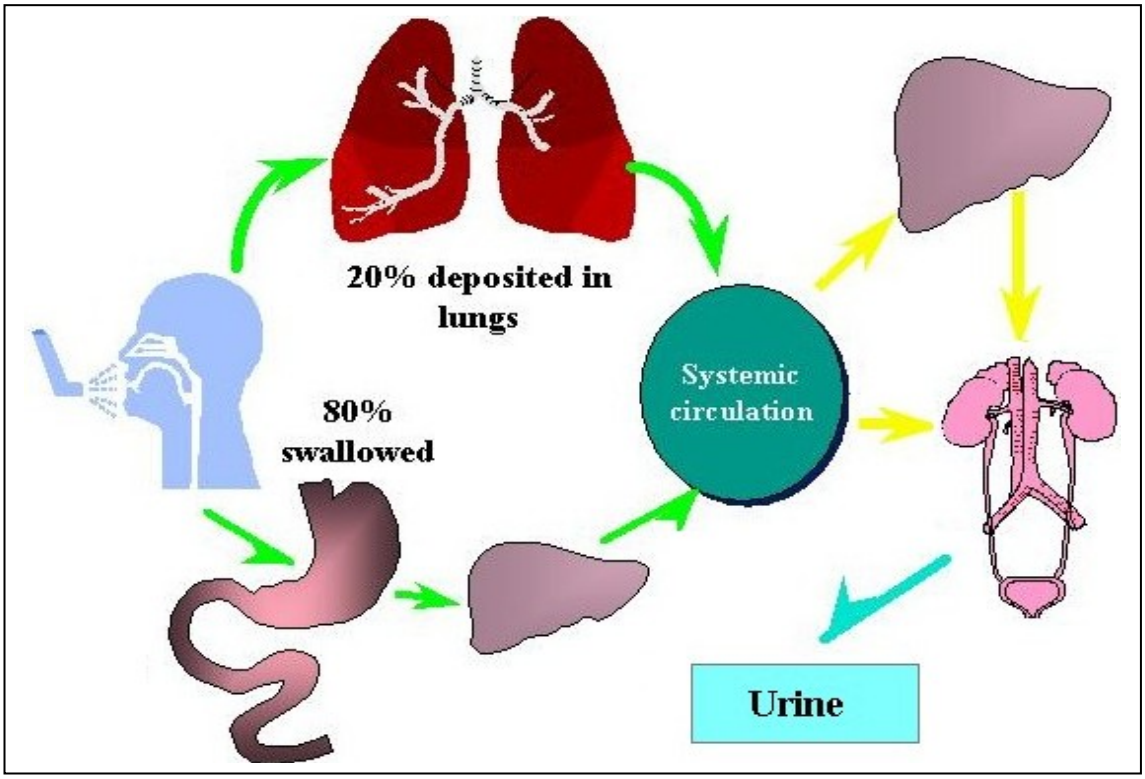


Figure 2.31 Pharmacokinetics of inhaled drug [Reproduced from (Chrystyn 2001).]

Using the lag time of the gastrointestinal absorption Hindle and Chrystyn (1992) developed a urinary pharmacokinetic method to determine the relative lung and systemic bioavailability of inhaled salbutamol. They showed that in the first 30 minutes after the oral administration of salbutamol, insignificant amounts of salbutamol were excreted in the urine, 0.18 (0.14) % of dose. This small amount is due to lag time between administration and absorption from the small intestine. On the other hand, they found significantly ($p<0.001$) more salbutamol was excreted in the urine over the first 30 minutes post inhalation, 2.06 (0.80) of the dose as shown in Figure 2.32. Using this method Hindle and Chrystyn (1992) reported a low intra-subject variability. The relative standard deviation for two volunteers was 6.4 % and 5.8 % (n=10). They therefore reported that an index of relative bioavailability can be obtained from the amount of salbutamol excreted in the first 30 minutes post inhalation. Furthermore, by the coadministratration of oral charcoal with salbutamol, urine and collecting urine over 24 hours then data concerning total lung deposition can be measured (Chrystyn et al, 1997).

The urinary pharmacokinetic method is very simple and non-invasive. The method has been extended to assess the relative lung bioavailability of inhaled sodium cromoglycate (Aswania et al. 1997; Aswania et al. 1999; Aswania and Chrystyn 2001; Aswania and Chrystyn 2002), nedocromil (Aswania et al. 1998), gentamicin (Al-Amoud et al. 2002), tobramycin (Barber 2002) and formoterol (Nadarassan et al. 2007).

The disadvantages of the pharmacokinetic methods are the need to differentiate between the swallowed and inhaled fraction of the inhaled dose. They do not identify dose deposition into different zones of the lungs and some assays do not have the sensitivity to measure the low concentrations (Chrystyn 2001).

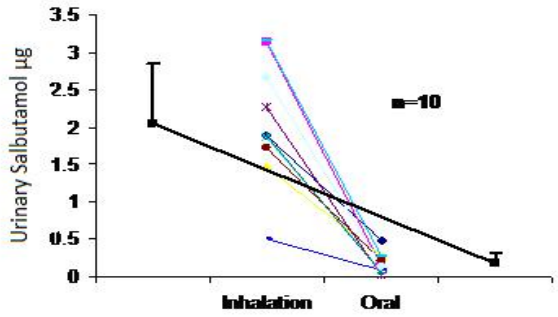


Figure 2.32 Mean and individual amounts of urinary salbutamol excreted during the first 30 minutes post inhalation and oral dosing (Hindle and Chrystyn 1992).

2.7.2.2 Gamma scintigraphy:

There are two types of gamma scintigraphy used in pulmonary imaging, two dimensional and three-dimensional imaging methods (Newman and Wilding 1999; Chrystyn 2000).

The two dimensional gamma scintigraphy (planer imaging) method usually uses 99m Technetium adhered to either the formulation or the drug molecules in the dosage form (physical attachment). The subjects inhale the combination and then use rapid imaging of a

radionuclide to identify the drug deposition in the lung following inhalation (Borgstrom and Newman 1993; Newman and Wilding 1999; Bondesson et al. 2003). The planar images obtained with this method may be insensitive to the relative deposition in the different zones of the lungs.

Three-dimensional imaging methods (SPECT and PET) have recently been introduced to overcome the disadvantage of planner imaging. SPECT (single photon emission computed tomography) is similar to two-dimensional gamma scintigraphy (physical attachment of the radiolabelling of the drug) except that the gamma camera rotates through 360^{o}C. This increases the data collection time. Hence, a very large dose is required. The dose may be 40 times larger than that required for the planar imaging and thus introduces formulation and preparation problems (Newman and Wilding 1999).

PET (Positron emission tomography) is a direct incorporation of a radiolabel into the drug molecule (chemical attachment). The ones recently used are positron emitters such as ^{11}C (short half life) or ^{18}F (long half life). The positron emitters used so far have short half-lives and the method is very expensive. ^{11}C has been introduced into triamcinolone acetonide and studies have highlighted the greater peripheral deposition when a spacer is attached to an MDI. This was mainly due to a substantial increase in the total amount of drug deposited in the lungs (13.6% with and 4.9% without the spacer). This technique has also been used for fluticasone (Berridge et al. 1998).

Gamma scintigraphy produces data of the total lung dose that is absorbed through the airways and cleared by mucociliary clearance. Since the former is the part of the dose that is responsible for the therapeutic action in the airways then gamma scintigraphy will overestimate the effective dose reaching the lung. The charcoal block method using urinary excretion of terbutaline (Borgstrom and Nilsson 1990) has been compared with total lung deposition measured by gamma scintigraphy (Borgstrom et al. 1992). The mean (SD) terbutaline excreted in the urine post inhalation with concurrent charcoal administration

was 21.1 (3.2) % of the nominal dose whilst gamma scintigraphy showed the total lung deposition to be 26.9 (3.8) %. The difference obtained is because part of the inhaled dose is cleared by mucociliary clearance. This fraction of the dose delivered to the lungs is identified by gamma scintigraphy but not by pharmacokinetic methods.

The long term safety and the expensive study costs are considered disadvantages of gamma scintigraphy (Chrystyn 2001). In addition, the labelling procedure involves manipulations of the formulations. Consequently, the radiolabelled formulation is different from the formulation in the original product although in-vitro tests are carried out to confirm similarity (Snell and Ganderton 1999). However, a previous report has shown that when using the Andersen Cascade Impactor the mass median aerodynamic diameter (MMAD) of a labelled drug was larger than the original product and that there was a difference in the homogeneity of the size distribution (Newman et al. 1982). Furthermore, particle size ranges should be quoted as amounts emitted rather than a percentage, and in-vitro determinations should use the same number of doses that were used in the scintigraphy study. Agencies such as the FDA are very cautious in using results from imaging studies for assessing bioequivalence (Mobley and Hochhaus 2001).

There are also non-radioactive assessment methods such as nuclear magnetic resonance imaging (MRI) and magnetic marker monitoring (MMM) but their use is not well established yet (Newman and Wilding 1999).

2.7.2.3 Pharmacodynamic method:

Clinical studies using spirometry or bronchoprovocation challenge have been used to identify the bioequivalence between two inhaled products (Eiser et al. 2001; Rodriguez-Carballeira et al. 2001). A method often used in the evaluation of the efficacy of inhaled drugs is the protective effect on methacholine or histamine induced bronchoconstriction (Tattersfield 1987; Britton et al. 1988). The inhalation of a short-acting β_2-agonist increases the provocative dose of inhaled methacholine or histamine from 1.1 to 3.9 times

(Casterline et al. 1976; Cockcroft et al. 1977). More recent studies have demonstrated that salbutamol increases the provocative dose of methacholine by 2.8 to 3.1 times (Wong et al. 1997; Seppälä et al. 1998). Most clinical studies that utilise spirometry as the primary outcome are carried out using measurements at the flat (plateau) portion of the dose-response relationship. For instance, a doubling of the therapeutic fluticasone inhaled dose has been shown to increase the peak expiratory flow rate (PEFR) by only 4.3 L min^{-1} from a baseline of almost 200 L min^{-1} (Dahl et al. 1993). Also for beclomethasone the FEV_1 (forced expiratory volume in the first second) increased by 0.18 L above baseline after 200 mg inhaled twice daily and by 0.21 L after 400 mg twice daily (Raphael et al. 1999). For the β_2-agonists the maximum response from therapeutic inhaled doses has been studied (Barnes and Pride 1983). It has been found that in normal subjects a maximum airway response to inhaled salbutamol was achieved with a cumulative dose of 110 µg. By contrast the dose required to produce a maximal bronchodilator response in asthmatic subjects was significantly higher and increased as the severity of bronchoconstriction increased (Barnes and Pride 1983). Newman et al. (1991) also demonstrated this in asthmatic subjects except that they also measured lung deposition using gamma scintigraphy. When these subjects inhaled radiolabelled salbutamol from a MDI and a MDI attached to a large volume spacer the total lung deposition was 12.3 and 23.1% (of the dose), respectively, but there was no difference in spirometry measurements (Newman et al. 1991). Also bronchoprovocation challenge cannot differentiate between different inhalation techniques due to the large variability of the method (Tomlinson et al. 2005). The inter-patient variability in clinical studies is high. Thus sensitivity to detect a difference is low and so a large number of subjects need to be studied (Barry and O'Callaghan 2003). Furthermore the bronchoprovocation agents may stimulate different receptors to those of the drug studied causing deterioration of lung function.

2.8 ß2-adrenoreceptor agonists

β2-adrenoreceptors are located in many tissues including the airways. Salbutamol and terbutaline are short acting β2-adrenoreceptor agonists. They stimulate β2-recetors located on the cell membrane of smooth muscles present in the airways resulting in bronchodilation and therefore providing relief for asthma sufferers and other conditions associated with airways obstruction.

2.8.1 Salbutamol sulphate

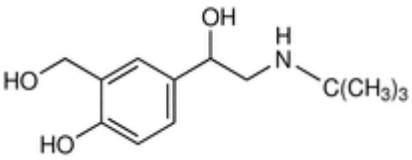


Figure 2.33 Molecular structure of Salbutamol (Sweetman, Martindale (Eds) 2009)

Salbutamol is a white, crystalline odourless powder with the chemical name a'-[(tert-Butylamino)methyl]-4-hydroxy-m-eylene-a.a'-[[1,1-dimethylethy]amino]methyl]-4-hydroxy-1,3-benzeedimethanol. It has molecular weight of 239.3 and is relatively polar, hydrophilic compound with basic properties. Salbutamol is soluble in 70 parts of water and in ethanol (750g/L) while slightly soluble in ether. The pharmacological activity is usually in the (R)-enantiomer, so far there is no evidence that (S)-enantiomer is harmful to the patient (Waldeck 2002). Due to salbutamol`s relatively polar and basic properties (Figure2.33), following inhalation passive tubular reabsorption does not occur, as ionisation of the drug is unaffected by changes in urine pH (Chrystyn, H., 1998). Once administered salbutamol is eliminated either as unchanged drug or as an inactive metabolite (Martin et al. 1971). Following oral administration salbutamol is absorbed from the gastrointestinal tract and has an absorption half life of 20 to 30 minutes (Goldstein et al. 1987). Peak plasma levels occur within 1.8 to 3 hours after oral administration (Martin, 1971; Goldstein, 1987), and 48% of the dose is eliminated as the metabolite, and 32% as unchanged salbutamol (Morgan et al. 1986). This is half the amount that is eliminated as unchanged drug following intravenous administration (64%). The low bioavailability

following oral administration of salbutamol is due to extensive first pass metabolism in the liver and gastrointestinal wall.

A study by Shenfield and colleagues found that after direct bronchial instillation of radio labelled salbutamol, 62% of the drug was eliminated as unchanged drug. They concluded that metabolism does not therefore occur in the lungs (Shenfield et al. 1976). Due to the rapid renal elimination of the portion of the drug absorbed through the lung, the peak urinary excretion of salbutamol is within 30 minutes post inhalation (Hindle and Chrystyn 1992).

2.8.2 Terbutaline sulphate

Terbutaline sulphate is a synthetic resorcinol derivative β_2-adrenergic agonist that is used as a bronchodilator in the treatment of asthma. It was first introduced in 1970s (Waldeck 2002; Sears and Lotvall 2005).

The Molecular Weight is 548.658 and the empirical formula is $(C_{12}H_{19}NO_3)_2.H_2SO_4$. Its chemical name is [(1RS)-1-(3,5-Dihydroxyphynyl)-2-(tert-butylamino)-ethanol] sulphate (2:1 salt) (British Pharmacopoeia 2005). Figure 2.34 shows the molecular structure of terbutaline sulphate.

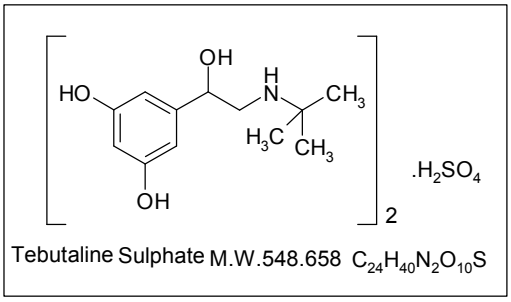


Figure 2.34 Molecular structure of terbutaline sulphate.

It is a hydrophilic white to grey-white crystalline powder, odourless or with a faint odour of acetic acid, soluble in water and 0.1N-hydrochloric acid, insoluble in chloroform and slightly soluble in methanol (United States Pharmacopeia 2002).

The commercially available terbutaline sulphate is a racemic mixture. Its melting point range differs according to the crystal type, as there are two types of the crystal form. Crystal form A has a melting point range from 268^0C to 271^0C and the crystal form B has a melting point range from 258^0C to 260^0C (Analytical Profiles 1990). Terbutaline sulphate is present in many dosage forms (inhalation, oral solution, injection and tablet) for prophylactic and acute bronchodilator treatment

Terbutaline sulphate is a β_2-adrenoreceptor agonist that is used as bronchodilator to open up constricted airways. Terbutaline sulphate is present in many dosage forms (inhalation, oral solution, injection, and tablet). After inhalation, the bronchodilating effect of terbutaline usually begins within 5 minutes and last for about 3 to 4 hours (Florey 1990). Orally administered terbutaline sulphate is usually absorbed from the gastrointestinal tract (Florey, 1990; (Sears and Lotvall 2005). The onset of action of orally administrated terbutaline is about 30 minutes and its duration of action is up to 8 hours (Martindale 2002). About 60% of the absorbed dose undergoes first pass effect metabolism to the sulphate ester (Sweetman and Britain 2002). Terbutaline is excreted in the urine as an inactive conjugate and the unchanged terbutaline. Its biological half life is 3 to 4 hours (Florey, 1990).

2.9 Summary

The literature highlights that the efficacy of inhaled therapy depends on the ability to deliver adequate aerosol in an optimal size-range to the lungs, which in turn depends on aspects of airway anatomy and physiology that will alter with age and disease status. The treatment of asthma, although based on regular use of corticosteroids as preventers, still relies heavily on the use of ß2-agonists such as salbutamol and terbutaline as reliever therapy. The management of COPD includes smoking cessation, the use of bronchodilators (ß2-agonists/antimuscarinics) and a combination of long acting ß2-agonist and corticosteroid as well as supplemental long-term oxygen therapy. COPD patients may

benefit from antibiotics and mucolytic drugs, especially during exacerbation, together with appropriate management of any associated cardiovascular disease.

All currently available DPIs rely on a patient's inspiratory effort to generate sufficient inspiratory flow that creates turbulent energy in the device for the dispersion and delivery of aerosol to the lungs. Each type of DPI has a different intrinsic resistance (due to design) against inspiratory flows, therefore, requires a specific inhalation technique for optimal aerosol generation and delivery to the lungs. Some COPD patients are not able to generate the optimal inspiratory flow through a DPI for efficient aerosol delivery to the lung, while others are not able to generate the threshold inspiratory flow required to lift the metered dose out of the device for delivery to the lungs. There is a link between the emitted fine particle dose, lung deposition and clinical response. Recently, the In-Check Dial has been introduced to identify a patient's inhalation rate through an inhaler and therefore the device to suit a patient's natural technique. Also study of ex vivo dose emission of DPIs using healthy volunteers could be carried out with the aid of the In-Check Dial.

Adaptation and modification of the compendia in-vitro methods enable the studies of patients simulated inhalation technique for various DPIs. The in vitro methods in combination with in vivo (gamma scintigraphy, pharmacokinetics and clinical) methods could be used for the determination of bioequivalence of inhaled products.

Lung deposition from an inhaler is dependent on the inhalation technique used. For an MDI a slow, deep and prolonged inhalation is required whilst for a DPI the inhalation manoeuvre should be fast from the start of the inhalation and sustained for a long as possible. The quality of the emitted dose for a DPI is dependent on inhalation flow and inhalation volume along with the ability to empty the dose out of the metering cup or capsule. For the latter reason patients may need to inhale each dose twice.

The studies in this thesis have been designed to identify the effect and influence of inhalation flow, inhalation volume and the number of inhalations for each dose. Four

different DPIs have been used to identify the minimum criterion of the inhalation manoeuvre to deliver a dose into the lungs of patients.

2.9.1 Criteria for the selection of the four different dry powder inhalers (DPIs)

Previously, an in-vitro study on the effects of inhalation manoeuvre on the emitted dose of tiotropium from a Handihaler (a single dose capsule) DPI has been performed. The results of the study have shown that dose emission was influence by inhalation flow and volume and that two inhalations were required for each dose (Al-Fadhl, 2005). The results of that study have, in part, generated a considerable interest for a similar study on multiple dose DPIs, which has never been done. At present the instructions (manufacturer's patient information leaflet) for using available multi-dose DPIs state only one inhalation per dose and patients should inhale as fast as they can. The criteria for the selection of the four different multiple dose DPIs used for the studies in this Thesis are as shown in Table 2.11.

Table 11 Criteria for the selection of the four different dry powder inhalers (DPIs)

Device	Company (Country) Licensee	Drugs available	Type
Accuhaler	GlaxoSmithKline (UK)	Salbutamol	Multiple unit-dose strip Low resistance
Clickhaler	ML Laboratories (UK)	Salbutamol	Multiple-dose reservoir Intermediate resistance
Easyhaler	Orion Pharma (Finland)	Salbutamol	Multiple-dose reservoir High resistance
Turbuhaler	AstraZeneca (Sweden)	terbutaline	Multiple-dose reservoir High resistance

Chapter 3

3 High Performance Liquid Chromatography (HPLC)

3.1 Introduction

Generally, chromatography is a separation technique in which the sample components, carried by the mobile phase are separated by adsorption-partition steps on a stationary phase. High-performance liquid chromatography (HPLC) comprises all liquid chromatographic techniques with the use of high pressure to force the liquid (mobile phase) through the stationary phase. The sample to be analysed is introduced in a small volume to the stream of mobile phase and is retarded by chemical or physical interactions with the stationary phase as it traverses the length of the column. Separation of analytes is based on their relative affinity for the stationary and mobile phases and selectivity is influenced by the column temperature and pH (Watson 2005).

Early forms of HPLC utilised polar stationary phases and non-polar mobile phases. This type of chromatography is known as normal phase chromatography. Most separations by HPLC nowadays utilise reversed-phase chromatography where a non-polar stationary phase is used in conjunction with a polar mobile phase (Hagan 1994). Using this system, polar analytes elute faster than non-polar analytes.

3.2 HPLC assay for aqueous salbutamol sulphate

In this study the HPLC assay for aqueous salbutamol sulphate was based on that published by Silkstone, (1999) with minor modifications to the mobile phase made by Richardson (2003). The modified mobile phase consisted of phosphate buffer: acetonitrile (80:20) adjusted to pH 2.5 with orthophosphoric acid and a flow rate of 1ml/minute used. This was further modified using 5mM potassium dihydrogen phosphate: acetonitrile (75:25), adjusted to pH 3 with orthophosphoric acid using the flow rate of 1ml/minute. The method was re-validated according to the International Conference on Harmonisation of Technical Requirements for registration of pharmaceuticals for human use guidelines (ICH 1994).

3.2.1 Chemicals

Salbutamol sulphate (Sigma), terbutaline sulphate (Sigma), bamethane sulphate (Sigma), potassium dihydrogen orthophosphate (BDH), orthophosphoric acid (BDH), acetonitrile (Fisher) and double distilled water were of HPLC grade.

Table 3.1 Instrumentation and HPLC conditions

Stationary phase:	Phenomenex Sphere Clone column 5µ ODS (2) 250×4.6mm set at temperature of 30^{o}C
Mobile phase:	Buffer: acetonitrile (75:25), the buffer was 5mM potassium dihydrogen orthophosphate buffer adjusted to pH3, with orthophosphoric acid. The mobile phase was filtered through a 45mm membrane filter (Millipore, Whatman Ltd, UK) and degassed under vacuum in an ultrasonic bath for 10 minutes prior to use.
Internal standard:	Bamethane sulphate 8000µg L^{-1}
Flow rate:	1 ml min^{-1}
Pump:	HP 1050 pumping system (Hewlett Packard, Germany)
Injector:	HP 1050 Autosampler with a 200µl loop
Detector:	UV- detector, set at wavelength 202 nm
Integrator:	A package using Prime software (HPLC Technology Ltd, UK)

3.2.2 Standards

Aqueous stock solutions of salbutamol sulphate and terbutaline sulphate were respectively prepared and stored in a refrigerator. From the respective stock solution, working standards were prepared by serial dilution using aqueous bamethane solution internal standard (8000µg/L) to yield nominal salbutamol sulphate and terbutaline sulphate concentrations of 250, 500, 750 and 1000, 1500 and 2000µg L^{-1} (w/v). The working solutions were stored in well closed, light resistant containers kept in a refrigerator prior to analysis.

3.2.3 Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtained test results which are directly proportional to the concentration (amount) of analyte in the sample. Calibration curves were obtained using six salbutamol sulphate standards between 250 and 2000µgL^{-1} with bamethane sulphate (8000µgL^{-1}) as an internal standard. Two injections were performed for each salbutamol sulphate standard. The peak area ratio of salbutamol to bamethane was plotted against the nominal concentration of salbutamol sulphate standard. A straight line was fitted to the data using linear regression. A representative plot described by the equation y=0.0003x+0.0006 (r^2=0.9996) is shown in Figure-3.1. Representative chromatograms are as shown in Figure 3.2. The detector response was shown to be linear over the range of 250 to 2000µgL^{-1} of salbutamol sulphate in aqueous sample containing 8000µgL^{-1} of bamethane with correlation coefficients of 0.9996.

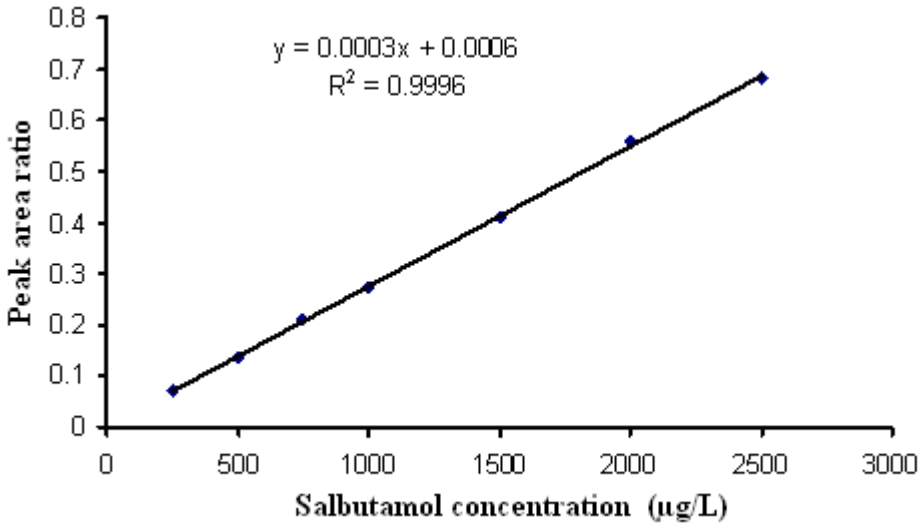


Figure 3.1 A representative calibration curve of the peak area ratio of salbutamol sulphate to bamethane sulphate against the concentration of salbutamol sulphate standard

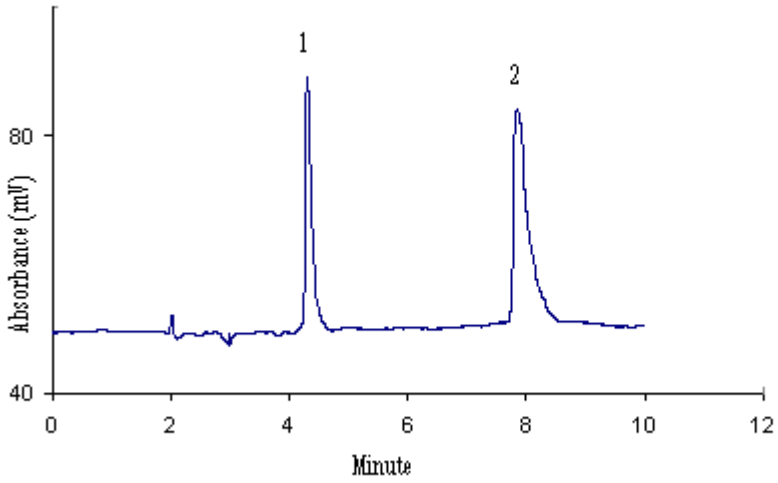


Figure 3.2 Typical chromatograms of (1) salbutamol sulphate aqueous standard (2000µg/L) and (2) bamethane sulphate aqueous standard (8000µgL^{-1}) injected under the following chromatographic conditions- Phenomenex Sphere Clone 5µ ODS (2) 250×4.6mm set at temperature of 30^{o}C; Mobile phase (25:75 v/v%) acetonitrile: 5mM potassium dihydrogen orthophosphate buffer adjusted to pH3, with orthophosphoric acid; flow rate 1.0 mLmin^{-1}; wavelength =202 nm; injection volume 50µL

3.2.4 Precision

This is a measure of the distribution of individual measurements around the mean. This parameter is generally assessed by repeated analysis of the same solution and expressed as the relative standard deviation (RSD) otherwise known as the coefficient of variation (CV). The lower the value the better is the assay performance. Six injections per salbutamol standard (concentration ranging from 250-2000 μgL^{-1}) were performed on the same day for intra-day precision of the assay, while inter-day precision of the assay was determined on different days under the same HPLC conditions for six days. Intra-day and inter-day variations, expressed as the relative standard deviation (RSD in peak area ratio, were calculated by dividing the standard deviation of the calculated concentrations by the mean concentration and multiplying by hundred. The mean of (RSD) intra-day assay variability, determined for the six (250-2000 μgL^{-1}) standard concentrations of salbutamol sulphate on six occasions, was 0.89. The inter-day assay variability, determined at the same six standard concentrations, using six replicate runs on different days was 1.68. The results are shown in Table 3.1.

Table 3.2 Precision of salbutamol sulphate assay (n=6)

Nominal concentration μgL^{-1}	Intra-day (RSD)%	Inter-day (RSD)%
250	2.21	0.99
500	0.55	2.22
750	0.73	1.18
1000	0.57	1.88
1500	0.55	1.15
2000	0.74	2.64
Mean	**0.89**	**1.68**

3.2.5 Accuracy

This describes the closeness of test results obtained by an analytical method to the true value (concentration) of the analyte. Three different concentrations (400, 800 and 1250μgL^{-1}) of salbutamol standards within the linear range of 250 to 2000μgL^{-1} were made and six chromatographs per concentration of these standards were performed. The accuracy of the assay was measured by comparing the mean observed concentration obtained from the linear regression, described by the equation y=0.0003+0.0006 to the nominal salbutamol concentration. The accuracy was evaluated by determining the percentage relative error (RE %) that is the difference between the true value (nominal concentration) and the observed concentration divided by the true value and multiplied by hundred. The results are as shown in Table 3.2. The accuracy is within the acceptable range of 100±2%.

Table 3.3 Accuracy of salbutamol sulphate assay (n=6)

Nominal concentration (μgL^{-1})	Mean observed concentration (μgL^{-1})	Accuracy (RE %)
400	401.6	0.4
800	794.4	-0.7
1250	1252.4	0.19

3.2.6 Limits of detection and quantitation

The ICH and FDA guidelines recommend the use of a method based on the signal-to-noise approach and a method based on the standard deviation of the response and the slope (liner regression line method) to determine the limit of quantitation (LOQ) and the limit of detection (LOD).

According to the method based on the signal-to-noise approach, the limit of detection (LOD) is the lowest concentration of analyte that can be detected but not quantified and it should be a value greater than 3:1 for the signal to noise ratio. The limit of quantitation

(LOQ) is the lowest concentration of analyte that can be measured with acceptable precision and accuracy by the assay and it should be a value greater than 10:1 for the signal to noise ratio. The limit of detection (LOD) and the limit of quantitation (LOQ) can be calculated from the mean of the slope and SD of the intercept of five calibration curves using the linear regression line method. The LOD is equal to 3.3 multiplied by the SD of the intercept of the linear regression line divided by its slope. The LOQ is equal to the 10 multiplied by SD of the intercept of the linear regression line divided by its slope. The linear regression line method was used here to determine LOD and LOQ.

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the mean and standard deviation (SD) of the intercept and slope of five salbutamol sulphate calibration curves. The LOD and LOQ with 50µL injection volume were 75.2µgL^{-1} and 228µg/L of salbutamol sulphate respectively.

3.2.7 Recovery

1ml of salbutamol sulphate standard (100,000µgL^{-1}) was added to a filter (type A/E) in a Petri dish and allowed to dry overnight. The filter was then transferred into a beaker containing 100ml of bamethane 8000µgL^{-1} (in distilled water); the beaker was covered with parafilm and left overnight. The resultant salbutamol sulphate solution was placed in sonic bath for three minutes and then filtered for HPLC assay. The procedure was repeated for ten different filters. The mean recovery of salbutamol sulphate from the filter following the HLPLC assay is 99.96 (n=10) with RSD=6.3%.

3.3 HPLC Assay for aqueous terbutaline sulphate

The HPLC assay conditions were as for salbutamol sulphate described in section 3.2.

3.3.1 Linearity

Calibration curves were obtained using six terbutaline sulphate standards between 250 and 2000µg/L with bamethane sulphate (8000µgL^{-1}) as an internal standard. Two injections

were performed for each terbutaline sulphate standard. The peak area ratio of terbutaline to bamethane was plotted against the concentration of terbutaline sulphate standard. A representative plot described by the equation y=0.0003x+0.0297 (r^2=09996) is as shown in Figure 2.3. Typical chromatograms of terbutaline sulphate standard concentration (1) and bamethane sulphate internal standard (2) are as shown in Figure 3.3.

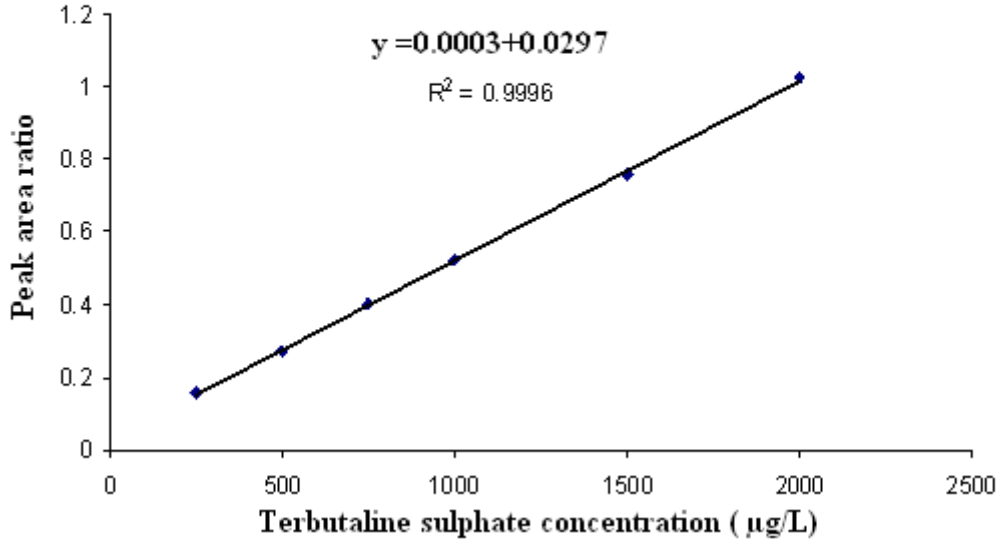


Figure 3.3 A representative calibration curve of the peak area ratio of terbutaline sulphate to bamethane sulphate against the concentration of terbutaline sulphate performed

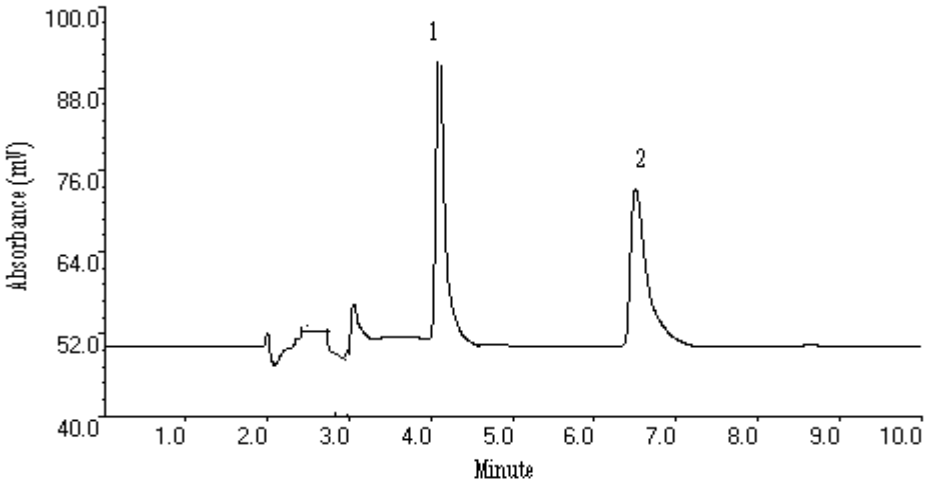


Figure 3.4 Typical chromatograms of (1) terbutaline sulphate aqueous standard (2000µg/L) and (2) bamethane sulphate aqueous standard (8000µg/L) injected under the following chromatographic conditions- Phenomenex Sphere Clone 5µ ODS (2) 250×4.6mm set at temperature of 30^{o}C; Mobile phase (25:75 v/v%) acetonitrile: 5mM potassium dihydrogen orthophosphate buffer adjusted to pH3, with orthophosphoric acid; flow rate 1.0 mL/min; wavelength=202nm; injection volume 50µL

3.3.2 Precision

This is a measure of the distribution of individual measurements around the mean. This parameter is generally assessed by repeated analysis of the same solution and expressed as the relative standard deviation (RSD) otherwise known as the Coefficient of Variation (CV). The lower the value the better is the assay performance. Six injections per terbutaline standard (concentration ranging from 250-2000µgL^{-1}) were performed on the same day for intra-day precision of the assay, while inter-day precision of the assay was determined on different day under the same HPLC conditions for six days. Intra-day and inter-day variations, expressed as the relative standard deviation (RSD) in peak height ratio, were calculated by dividing the standard deviation of the calculated concentrations by the mean concentration and multiplying by hundred. The mean of intra-day (RSD) assay

variability, determined for the six standard concentrations of terbutaline sulphate on six occasions, was 0.96. The mean of inter-day (RSD) assay variability, determined at the same three concentrations, using six replicate runs on different days was 1.59. The results are shown in Table 3.3.

Table 3.4 Precision of terbutaline sulphate Assay (n=6)

Nominal concentration	Intra-day	Inter-day
μgL^{-1}	(RSD)%	(RSD)%
100	2.4	1.47
200	1.13	3.84
250	1.21	0.98
500	0.92	1.90
750	0.32	1.17
1000	0.36	0.07
1500	0.35	1.68
Mean	**0.96**	**1.59**

3.3.3 Accuracy

This describes the closeness of test results obtained by an analytical method to the true value (concentration) of the analyte. Three different concentrations (300, 800 and 1200μgL^{-1}) of terbutaline sulphate standards within the linear range of 250 to 2000μgL^{-1} were made and six chromatographs per concentration of these standards were performed. The accuracy of the assay was measured by comparing the observed concentration obtained from the linear regression, described by the equation y=0.0005x+0.0297 to the nominal concentration of terbutaline sulphate. The accuracy was evaluated by determining the percentage relative error (RE %) and the results are as shown in Table 3.4. The accuracy is within the acceptable range of 100±2%.

Table 3.5 Accuracy of terbutaline sulphate assay (n=6)

Nominal concentration μgL^{-1}	Mean observed concentration μgL^{-1}	Accuracy (% RE)
300	299.2	-0.26
800	805	0.62
1200	1195.7	-0.36

3.3.4 Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the mean and standard deviation (SD) of the intercept and slope of five terbutaline sulphate calibration curves. The LOD and LOQ with 50µL injection volume were 45.9μgL^{-1} and139μgL^{-1} of terbutaline sulphate respectively.

3.3.5 Recovery

1ml of terbutaline sulphate standard (100,000μgL^{-1}) was added to a filter (type A/E) in a Petri dish and allowed to dry overnight. The filter was then transferred into a beaker containing 100ml of Bamethane in distilled water (8,000μgL^{-1}); the beaker was covered with parafilm and left overnight. The recovered terbutaline sulphate solution was placed in a sonic bath for three minutes and filtered for HPLC assay. The procedure was repeated for ten different filters. The mean recovery of terbutaline sulphate from the filter following the HLPLC assay was 99.86 (n=10) with RSD=4.3%.

3.4 Summary

The results show the aqueous HPLC assay methods for salbutamol sulphate and terbutaline sulphate were precise, accurate and sensitive. The relative standard deviations (RSD) for both assays were low (in the range 0.89 to 1.68). Also the assays both showed very little deviation from the nominal concentration when the concentrations of salbutamol sulphate

and terbutaline sulphate were calculated from the chromatographic peak area ratio. The accuracy is within acceptable range 100±2%.

The LOD and LOQ with 50µL injection volume were calculated to be: 75.2µgL^{-1} and 228µgL^{-1} (salbutamol sulphate) and 45.9µgL^{-1} and 139µgL^{-1} (terbutaline sulphate) these were well below the expected concentrations for samples to be analysed in this study. The LOD and LOQ in agreement with those obtained for salbutamol (Richardson, 2003).

Therefore, the methods could be used to determine the amounts of salbutamol and terbutaline in aqueous samples.

Chapter 4

4 In vitro determination of dose emission of dry powder inhalers (DPIs) at different inhalation flows using low and high inhalation volumes

4.1 Introduction

Inhalation of aerosolised drugs has been widely recognised as the therapy of choice for asthma and chronic obstructive pulmonary disease (COPD). Dry powder inhalers (DPIs) require an internal turbulent energy to be generated inside the device during an inhalation to lift the powder formulation from the metering system and disperse the emitted dose that contains particles with the potential for lung deposition. This internal turbulent energy is created by the interaction between the inhalation profile generated from the patient's inspiratory effort and the intrinsic resistance of the inhaler device (Chrystyn, 2003). Thus, aerosol generation within DPIs and the release of a dose for delivery to the lungs of patients are dependent on patient variability such as the inhalation flow, its acceleration rate, the inhalation volume and the number of inhalations per dose.

The pharmaceutical performance of inhaled products can be characterised by the emitted dose, fine particle dose and its aerodynamic particle size distribution. The efficacy and safety of an inhaled product are related to these parameters. In addition, they are often used to predict lung deposition and systemic delivery. The total emitted dose from a DPI can be measured in vitro using the uniformity of dose sampling apparatus described by the Pharmacopoeias (USP, 2009; EP, 2007; BP, 2008). According to these Pharmacopoeias, DPIs should be tested using a constant airflow drawn from a vacuum pump corresponding to a pressure drop of 4kPa across the inhaler using an inhalation volume of 4L. However, patients inhale at varying flows and volumes through DPIs. Furthermore, when patients inhale through a DPI, the inhalation volume is less than 4L, for example, it has been reported that the inhaled volume of asthmatic and COPD patients when using a DPI is about 2L (Hawskworth, et al., 2000). Also studies have shown that DPIs operate effectively at peak inhalation flows >30 $Lmin^{-1}$ and that the optimum flows for DPIs in

terms of the total emitted dose and fine particle dose is >60 $Lmin^{-1}$ (Bisgaard et al. 1998; Nielsen et al. 1998). Other studies have shown that some patients, especially those with COPD (Broeders et al. 2003) and children with severe asthma (Pedersen et al, 1990) were not able to generate the minimum flows through a DPI that is required to generate an emitted dose with the required characteristics for lung deposition. It is possible that these patients may require two inhalations in order to maximise the generation and the release of a dose for delivery to the lungs. At present unless the DPI is presented as a single dose capsule then patient information leaflets instruct that patients should inhale once per metered dose.

Since DPIs are tested using specific inhalation criteria that do not represent that of patients then it is important to assess the effects and influence of inhalation flow, inhalation volume and the number of inhalations. Hence, this study was designed to determine the dose emission from the Accuhaler®, the Clickhaler®, and the Easyhaler®, all containing salbutamol sulphate and the Turbuhaler®, containing terbutaline sulphate, following one and two inhalations at varying inhalation flows (10-60 L/min), using inhalation volumes of 2 and 4L .

The aims of this study were to use in-vitro studies to:

- Determine the effects of different inhalation rates and inhalation volume on the emitted dose from the Accuhaler®, the Easyhaler®, the Clickhaler® and the Turbuhaler®.
- Identify if there is a difference in the emitted dose from the above mentioned inhalers, when inhaling once or twice per dose.

4.2 Method

4.2.1 Instrumentation and inhaler devices

DPI sampling apparatus (Copley Scientific Ltd, UK)

A/E fibre glass filter discs 47 mm (Pall Corporation, New York, USA).

Vacuum pump GAST 1023-703QER56X (Brook Crompton, UK).

Flow meter (Model DFM, Copley Scientific Ltd, UK)

Integrated mass flow meter-pressure transducer (MKS Instruments, USA)

Parafilm M Laboratory parafilm (American National Can, USP)

Inhaler devices: Ventolin® Accuhaler® [ACC] containing salbutamol sulphate 200 µg per dose (GlaxoSmithKline); Clickhaler® [CLICK] containing salbutamol sulphate 114 µg per dose; Easyhaler® [EASY] containing salbutamol sulphate 200 µg per dose (Orion Pharma, Finland) and Bricanyl® Turbuhaler® [TBH] containing terbutaline sulphate 500 µg per dose, (Astra Zeneca, UK).

4.2.2 Procedure

The duration of inspiration (inhalation time) was set to allow inhalation volumes of 2 and 4L using the relation described in the USP (2005).

Time **T = (60 sec x V) / Q**

T = duration of inspiration consistent with withdrawal of air from the inhaler

Q = inhalation rate

V = 2L or 4L to be drawn through the inhaler

Table 4.1 Calculated inhalation time (sec)

Table 4.1 Calculation of inhalation time

Inhalation flow ($L\ min^{-1}$)	Time T (sec) 2L	Time T (sec) 4L
10	12	24
20	6	12
30	4	8
40	3	6
60	2	4

The emitted dose from each type of inhaler was measured using a DPI sampling apparatus as shown in Figure 4.1. The inhaler device was inserted into a mouthpiece adapter in an air tight chamber aligned along the horizontal axis with the sampling tube. The other side of the chamber was connected to the flow meter (Model MKS). A tube (T1) from the chamber was connected to one end of the pressure transducer and another tube (T2) from the sampling unit was connected to the other end of the pressure transducer as shown in Figure 4.1. This enabled pressure drop across the device to be monitored.

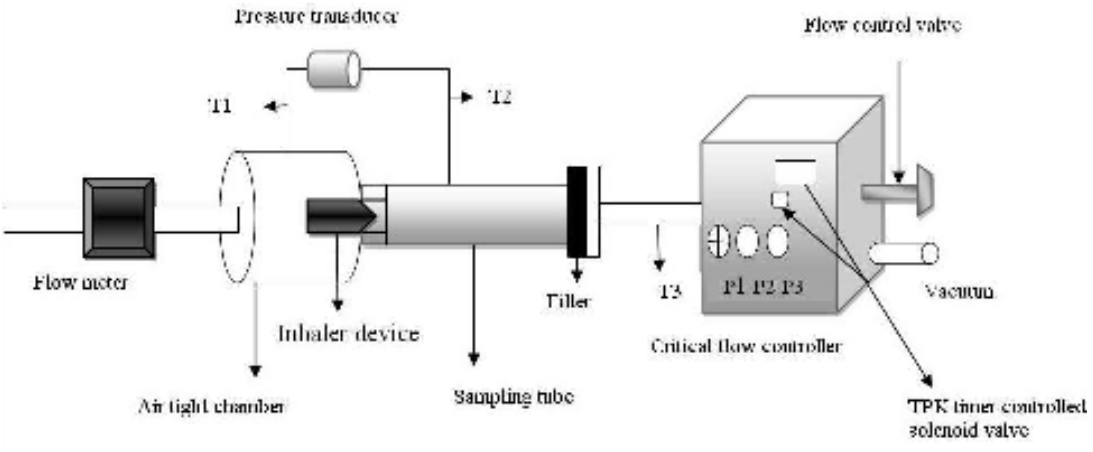


Figure 4.1 A Schematic diagrams of dose emission apparatus

The sampling apparatus was then connected to a critical flow controller (Model TPK) via a tube T3. The critical flow controller contains a two-way solenoid valve which controls the time and the pressure of the airflow. The two-way valve enabled air to be drawn through the mouthpiece of the inhaler at a set flow by a vacuum pump. The inhalation flow through the mouthpiece of each type of inhaler was set at 10, 20, 30, 40 and 60 L min^{-1} with corresponding calculated flow-durations as shown in Table 4.1 to allow the inhaled volumes of 2L and 4L respectively to be drawn through the inhaler (BP, 2005; USP, 2005). Critical (sonic) flow was ensured during each operation (absolute pressure ratio P3/P2 should be less or equal to 0.5).

Ten separate inhalers of each inhaled products were used in the study. For each determination a single dose was used. Each type of inhaler was loaded according to the instructions in the patient information leaflet and inserted into the dose sampling tube. Then the TPK timer controlling the solenoid valve was activated once to discharge the metered dose into the sampling tube at each set inhalation flow. Following discharge the inhaler device was flushed to waste at flow of 90 L min^{-1} for sixteen seconds to remove any remaining powder before proceeding to the next determination. The sampling tube and the filter were rinsed with standard aqueous bamethane solution (8000 μgL^{-1}) and the resultant solution was placed in a sonic bath for three minutes. Preliminary analysis (sections 3.2.8 and 3.3.5) revealed that this procedure removes the entire entrained drug on the filter. All the solutions were collected and made up to a volume of 50, 50, 100, 100, and 100ml, for the flow of 10, 20, 30, 40, and 60 L min^{-1}, respectively. The amount of active drug collected on the filter and deposited on the wall of the tube was determined by high liquid performance chromatography (HPLC) described in chapter 3.

This procedure was repeated for each determination using two inhalations per metered dose, except that the TPK timer controlling the solenoid valve was activated twice to obtain two separate discharges of powder into the sampling tube at each set inhalation flow.

A total of ten doses (randomised), three at the beginning, four in the middle and three drawn at the end of the lifetime of each type of inhaler as shown in the Table below, were analysed at each inhalation flow following one and two inhalations per dose for 2L and 4L inhaled volume respectively.

Turbuhaler label claim 100 doses	Easyhaler and Clickhaler 200 doses	Accuhaler 60 doses	Discharge
1	1	1	waste
2, 3, 4	2, 3, 4	2, 3, 4	collect
5-48	5-98	5-28	waste
49, 50, 51, 52	99, 100, 101, 102	29, 30, 31, 32	collect
53-97	103-197	33-57	waste
98, 99, 100	198, 199, 200	58, 59, 60	collect

4.2.3 Analysis of data

The emitted dose as determined from the dose sampling unit was calculated as μg per dose. Since the label claims of the studied inhalers differ, the emitted dose from each inhaler was expressed as a percentage of the nominal dose (label claim).

4.2.4 Statistical analysis

SPSS version 15.0 software (SPSS Inc., Chicago, USA) was used for the statistical analysis. A two-way analysis of variance (ANOVA) with the application of the General Linear Model Univariate was used to determine any significant differences in the emitted dose from four different inhalers under different flows. Also the statistical comparisons of the emitted dose between two different inhalation volumes as well as between one and two inhalations for each metered dose under the same flows were made. The mean difference

(95% confidence interval) was calculated and a probability value of $p<0.05$ was considered being significant.

4.3 Results

The individual emitted dose from the Accuhaler, Easyhaler, Clickhaler and Turbuhaler used in this study was expressed as a percentage of the nominal dose (the label claim). The individual emitted dose, mean (n=10), SD and RSD of the total emitted dose from each dry powder inhalers (DPIs) at varying inhalation flows (10-60 $Lmin^{-1}$) following one and two inhalations using 2L and 4L inhaled volume respectively are shown in Tables 4.2 to 4.9. A summary of the total emitted dose, expressed as % nominal dose from the four dry powder inhalers at varying inhalation flows (10-60 $Lmin^{-1}$) using 2L and 4L inhalation volumes following one and two inhalations per metered dose respectively is shown in Table 4.10. Figures 4.2 to 4.5 describe the variation of the emitted dose from the Accuhaler, Easyhaler, Clickhaler and Turbuhaler with respect to the inhalation flow under the same inhalation volumes and the number of inhalations.

Tables 4.11 and 4.12 show the statistical comparison of the emitted dose (% nominal dose) from four different dry powder inhalers at varying inhalation flows (10-60) $Lmin^{-1}$ using a 2L and a 4L inhaled volume respectively. This highlights the effect of the inhalation flow on the emitted dose from the inhalers.

Table 4.2 Emitted dose of salbutamol sulphate, expressed as % nominal dose, from the Accuhaler (salbutamol sulphate 200 µg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 2L inhaled volume

	Emitted dose of salbutamol sulphate (% nominal dose)									
Inhalation flow (L min^{-1})	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose										
1	49.37	53.30	32.78	51.06	79.03	81.65	63.35	68.39	87.35	89.39
2	50.27	52.11	49.14	65.16	61.4	61.4	67.39	67.39	87.65	87.65
3	53.78	51.70	63.14	80.69	62.38	64.97	76.91	76.91	84.02	84.02
4	48.78	61.69	50.78	58.64	71.3	71.3	73.73	73.73	88.4	88.4
5	53.98	58.10	65.28	66.59	62.51	67.45	61.03	62.12	83.1	86.61
6	43.68	58.62	56.78	67.93	66.28	68.39	71.38	71.38	87.71	90.34
7	49.62	56.33	66.75	67.79	67.68	69.43	97.5	97.5	83.58	88.7
8	52.64	52.91	43.93	57.02	73.42	82.67	67.88	72.57	81.92	84.52
9	50.79	55.13	50.2	55.72	63.59	80.7	81.33	86	78.28	80.48
10	46.76	57.18	47.57	48.18	69.63	74.47	65.56	77.88	75.57	75.86
Mean	49.97	55.71	52.64	61.88	67.72	72.24	72.61	75.39	83.76	85.60
SD	3.17	3.26	10.56	9.63	5.69	7.79	11.07	10.72	3.39	3.14
RSD	6.34	6.52	14.82	16.14	11.40	14.78	12.52	13.30	8.60	9.07

Table 4.3 Emitted dose of salbutamol sulphate, expressed as % nominal dose, from the Accuhaler (salbutamol sulphate 200 µg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 4L inhaled volume

Inhalation flow (L min^{-1})	**Emitted dose of salbutamol sulphate (% nominal dose)**									
	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose										
1	56.86	53.88	57.47	63.68	74.55	74.55	65.11	73.16	84.48	84.48
2	37.45	57.22	50.38	54.52	81.57	81.57	66.32	66.32	85.67	94.52
3	54.13	62.90	63.6	67.96	70.76	70.76	95.97	99.63	80.28	85.33
4	50.45	61.16	50	53.55	75.24	77.8	74.1	74.1	88.73	88.73
5	62.03	59.94	58.77	60.61	75.93	79.56	75.21	75.31	78.19	78.19
6	56.49	58.44	41.56	42.61	75.79	81.43	78.71	78.95	82.56	82.56
7	54.62	63.84	64.3	73.07	75.01	75.36	84.09	84.31	79.19	79.19
8	49.78	59.46	65.87	71.77	71.81	71.81	67.96	68.65	83.71	83.71
9	60.79	54.84	65.21	65.21	76.77	76.77	79.18	79.18	77.23	77.23
10	57.34	60.26	75.11	75.11	79.86	79.86	76.31	76.31	76.85	76.85
Mean	53.99	59.19	59.23	62.81	75.73	76.95	76.30	77.59	81.69	83.08
SD	7.00	3.21	9.75	10.20	3.24	3.89	9.76	9.87	3.80	5.47
RSD	12.96	5.42	14.03	14.49	4.26	4.77	12.06	12.00	4.89	6.78

Table 4.4 Emitted dose of salbutamol sulphate, expressed as % nominal dose, from the Easyhaler salbutamol sulphate (200 µg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 2L inhaled volume

Inhalation flow (L min^{-1})	**Emitted dose of salbutamol sulphate (% nominal dose)**									
	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose										
1	34.72	68.28	63.17	72.14	69.09	75.38	75.49	86.25	97.34	111.28
2	44.19	48.49	64.94	73.28	77.88	84.31	77.65	90.13	68.9	81.06
3	31.35	50.65	60.35	69.61	76.25	82.11	85.79	96.06	85.71	97.41
4	36.49	49.74	63.14	68.1	81.48	89.36	76.9	87.67	73	86.35
5	35.05	51.62	72.25	77.65	76.25	86.23	71.8	94.28	68.58	83.6
6	33.79	63.11	67.95	123.56	74.7	83.39	79.96	102.25	77.41	93.4
7	40.12	47.28	61.48	67.72	65.47	72.96	75.12	86.18	69.47	85.63
8	37.96	62.68	76.44	81.09	80	85.89	72.28	81.64	78.69	94.34
9	34.08	52.33	76.69	82.88	67.72	75.77	71.06	79.53	80.69	99.6
10	36.79	49.13	77.06	83.41	61.95	70.25	78	89.74	74.7	91.01
Mean	36.45	54.33	68.35	79.94	73.08	80.57	76.41	89.37	77.45	92.37
SD	3.64	7.44	6.69	16.44	6.59	5.68	4.63	7.20	9.42	9.53
RSD	9.99	13.70	9.29	7.96	9.10	8.13	5.76	7.59	11.54	9.74

Table 4.5 Emitted dose of salbutamol sulphate, expressed as % nominal dose, from the Easyhaler (salbutamol sulphate 200 µg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 4L inhaled volume

Inhalation flow (L min^{-1})	**Emitted dose of salbutamol sulphate (% nominal dose)**									
	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose										
1	33.9	43.19	67.05	71.23	70.04	80.12	70.18	82.57	79.91	96.12
2	37.4	48.63	68.44	72.68	61.08	83.37	79.11	92.43	74.97	91.37
3	42.6	51.38	82.74	88.13	79.63	88.5	82.90	94.46	79.86	96.12
4	34.19	43.28	82.97	94.41	69.14	78.52	84.20	95.65	73.46	91.33
5	41.84	51.51	55.18	61.77	72.49	83.04	89.61	100.29	86.76	105.28
6	33.29	40.62	87.27	92.61	91.28	101.63	74.89	88.65	82.48	99.49
7	36.62	43.61	70.45	76.62	93.46	104.05	73.05	84.12	90.08	107.67
8	33.94	45.62	82.87	94.43	75.49	85.84	76.14	89.69	78.38	94.88
9	41.09	38.17	70.38	75.02	70.79	79.56	74.55	88.80	90.68	108.33
10	35.48	45.12	70.95	77.10	64.69	75.54	81.05	93.76	80.66	94.95
Mean	37.04	45.11	73.83	80.40	74.81	86.02	78.57	91.04	81.72	98.55
SD	3.57	4.35	9.89	11.29	10.59	9.43	6.21	5.61	6.20	6.64
RSD	9.64	9.65	9.29	7.96	9.10	8.13	5.76	7.59	11.54	9.74

Table 4.6 Emitted dose of salbutamol sulphate, expressed as % nominal dose, from the Clickhaler (salbutamol sulphate 114μg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 2L inhaled volume

Inhalation flow (L min^{-1})	**Emitted dose of salbutamol sulphate (% nominal dose)**									
	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose										
1	28.02	39.67	45.55	56.13	38.99	45.22	48.85	57.86	82.22	88.8
2	25.65	30.92	44.06	50.91	54.78	54.78	56.80	62.37	93	101.52
3	28.92	32.71	39.66	43.42	60.44	60.44	64.56	69.74	91.53	96.74
4	27.5	31.51	57.43	58.06	52.89	52.89	56.93	63.57	91.92	97.84
5	27.8	34.46	36.32	36.32	67.56	67.56	57.74	65.05	91.44	96.17
6	23.52	28.58	41.06	41.06	51.63	51.63	63.28	68.80	82.62	87.24
7	24.48	30.08	41.71	41.71	51.06	51.06	60.68	66.01	77.08	81.84
8	35.17	25.80	55.76	63.97	39.07	39.07	64.97	69.47	77.99	82.56
9	27.05	25.04	51	59.40	46.32	46.32	74.22	80.40	79.95	85.59
10	32.68	31.30	52.93	60.22	46.18	46.18	80.42	85.26	76.7	81.94
Mean	28.08	31.01	46.55	51.12	50.89	51.52	62.85	68.85	84.45	90.02
SD	3.55	4.21	7.28	9.76	8.90	8.16	7.08	6.30	6.58	7.25
RSD	12.62	13.59	14.83	19.10	17.49	16.79	14.47	12.02	8.02	8.22

Table 4.7 Emitted dose of salbutamol sulphate, expressed as % nominal dose, from the Clickhaler (salbutamol sulphate 114µg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 4L inhaled volume

Inhalation flow (L min^{-1})	**Emitted dose of salbutamol sulphate (% nominal dose)**									
	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose1	23.62	24.61	42.22	47.21	68.48	73.36	76.58	81.77	77.89	84.63
2	28.85	35.87	50.32	57.72	54.17	60.86	49.59	54.04	100.24	104.89
3	34.33	37.21	46.68	55.31	59.59	65.25	67.81	74.85	78.34	83.44
4	30.32	38.32	47.87	54.37	54.7	60.08	63.88	68.97	81.44	86.40
5	32.12	42.85	44.22	50.29	53.19	62.06	63.51	68.49	90.76	95.62
6	23.61	33.55	59.25	66.27	50.01	53.93	64.96	69.74	82.73	92.19
7	32.21	27.38	46.74	53.27	49.99	53.83	66.21	71.4	101.51	106.96
8	24.25	27.02	61.77	67.38	52.46	55.92	75.17	80.55	80.48	86.22
9	22.71	25.41	59.36	65.13	50.12	54.39	75.39	80.97	90.19	94.69
10	25.94	34.62	53.34	80.17	53.16	57.76	67.57	72.73	84.04	90.50
Mean	27.80	32.68	51.18	59.71	54.59	59.74	67.07	72.35	86.76	92.55
SD	4.29	6.22	6.91	9.93	5.66	6.46	8.36	8.70	9.08	8.64
RSD	15.42	19.04	12.80	16.64	10.41	10.30	11.76	11.34	9.93	8.84

Table 4.8 Emitted dose of terbutaline sulphate, expressed as % nominal dose, from the Turbuhaler (terbutaline sulphate emitted dose 500μg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 2L inhaled volume

Inhalation flow (L min^{-1})	**Emitted dose of terbutaline sulphate (%nominal dose)**									
	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose										
1	25.36	50.25	40.04	52.18	51.78	46.06	47.07	47.73	76.25	77.39
2	20.67	49.61	36.72	52.89	52.76	42.2	51.62	59.178	63	63.11
3	28.69	49.64	38.35	54.99	54.5	43.97	54.55	54.85	66.41	69.58
4	31.47	41.85	33.08	54.32	54.32	35.56	55.31	58.06	63.67	69.41
5	21.25	38.79	53.16	52.23	52.23	55.92	53.08	53.33	63.94	69.28
6	21.07	47.01	44.71	47.7	46.74	51.83	57.32	57.32	64.12	64.12
7	27.25	43.29	50.34	40.63	40.63	55.82	57.87	57.9	64.96	66.29
8	23.13	40.38	52.63	42.3	40.87	61.8	50.23	50.63	62.39	62.39
9	25.18	36.71	49.63	37.39	37.39	56.55	50.73	50.73	62.93	62.93
10	20.02	45.15	62.14	50.61	50.61	69.82	51.77	51.88	64.4	64.4
Mean	24.41	44.27	46.08	48.52	48.18	51.95	52.96	54.16	65.21	66.89
SD	3.86	4.84	9.05	6.58	6.35	10.17	3.36	3.89	4.04	4.64
RSD	15.80	10.92	18.62	12.67	12.98	19.56	6.33	7.23	6.21	6.55

Table 4.9 Emitted dose of terbutaline sulphate, expressed as % nominal dose, from the Turbuhaler (terbutaline sulphate emitted dose 500µg / nominal dose) at different inhalation rates following one and two inhalations per metered dose using a 4L inhaled volume

Inhalation flow (L min^{-1})	**Emitted dose of terbutaline sulphate (%nominal dose)**									
	10		20		30		40		60	
No. of inhalations	one	two	one	two	one	two	one	two	one	two
Dose										
1	22.11	23.79	55.78	56.06	42.87	44.6	41.71	42.39	70.75	70.75
2	34.93	36.13	43.08	43.08	53.95	55.16	59.55	60.56	74.54	76.59
3	32.52	45.27	48.36	49.32	49.01	49.85	60.82	62.15	70.32	70.32
4	41.59	37.79	46.87	46.96	50.07	50.17	61.89	62.61	70.31	72.8
5	20.47	46.28	46.56	49.89	49.2	50.37	46.27	48.67	67.33	69.27
6	42.75	22.98	50.1	50.22	48.34	49.79	67.33	67.97	70.28	73.44
7	23.05	33.58	43.53	43.53	47.92	49.23	63.99	64.4	74.6	77.35
8	36.39	40.97	48.92	49.38	61.59	61.59	55.71	56.48	74.98	77.98
9	34.13	39.82	47.89	48.4	54.68	55.64	59.19	59.82	72.97	74.97
10	31.65	33.45	49.42	49.51	55.84	55.84	55.83	56.32	71.4	72.3
Mean	31.96	36.01	48.05	48.64	51.35	52.22	57.23	58.14	71.75	73.58
SD	7.83	7.92	3.58	3.66	5.24	4.91	8.33	8.07	2.61	3.21
RSD	24.49	22.01	7.45	7.53	10.21	9.20	13.72	13.11	3.44	4.15

Table 4.10 Mean (SD) total emitted dose, as % nominal dose from the four different dry powder inhalers following one and two inhalations per metered dose for 2L and 4L inhalation volumes

	Mean (SD) total emitted dose (% nominal dose) following one inhalation							
Device	Accuhaler		Easyhaler		Clickhaler		Turbuhaler	
Inhalation volume	2L	4L	2L	4L	2L	4L	2L	4L
Inhalation flow ($L\ min^{-1}$)								
10	49.97 (3.2)	53.99 (7.0)	36.45 (3.6)	37.04 (3.6)	28.08 (3.6)	27.80 (4.3)	24.41 (3.9)	31.96 (7.8)
20	52.64(10.6)	59.23 (9.6)	68.35 (6.7)	73.83 (9.9)	46.55 (7.3)	51.18 (6.9)	46.08 (9.1)	48.05 (3.6)
30	67.72 (5.7)	75.73 (3.2)	73.08 (6.6)	74.81 (10.6)	50.89 (8.9)	54.59 (5.7)	48.18 (6.4)	51.35 (5.2)
40	72.61(11.1)	76.30 (9.8)	76.41 (4.6)	78.57 (6.2)	62.85 (7.1)	67.07 (8.4)	52.96 (3.4)	57.23 (8.3)
60	83.76 3.4)	81.69 (3.8)	77.45 (9.4)	81.72 (6.6)	84.45 (6.6)	86.76 (9.1)	65.21 (4.0)	71.75 (2.6)
	Mean (SD) total emitted dose (% nominal dose) following two inhalations							
Device	Accuhaler		Easyhaler		Clickhaler		Turbuhaler	
Inhalation volume	2L	4L	2L	4L	2L	4L	2L	4L
Inhalation flow ($L\ min^{-1}$)								
10	55.71(3.3)	59.19(3.2)	54.33(7.4)	45.11(4.4)	31.01(4.2)	32.68(6.2))	44.27(4.8)	36.01(7.9)
20	61.88(9.6)	62.81(10.2)	79.94(16.4)	80.40(11.3)	51.12(9.8)	59.71(9.9)	48.52(6.6)	48.64(37)
30	72.24(7.8)	76.95(3.9)	80.57(5.7)	86.02(9.4)	51.52(8.2)	59.74(6.5)	51.95(10.2)	52.22(4.9)
40	75.39(10.7)	77.59(9.9)	89.37(7.2)	91.04(5.61)	68.85(7.1)	72.35(8.7)	54.16(3.9)	58.14(8.1)
60	85.60(3.1)	83.08(5.5)	92.37(9.5)	98.55(6.6)	90.02(7.3)	92.55(8.6)	66.89(4.6)	73.58(3.2)

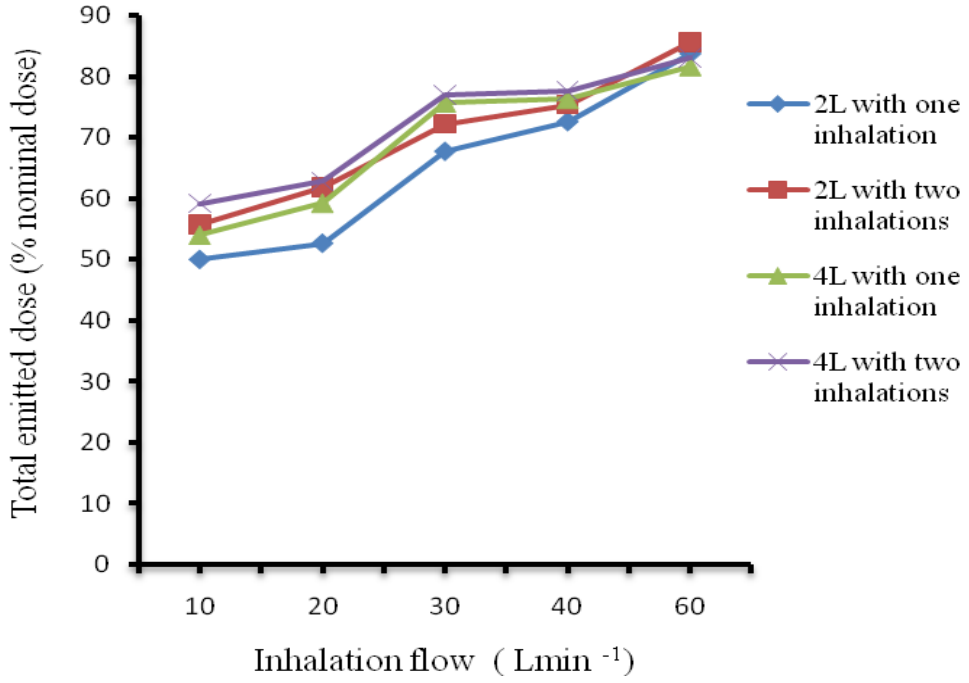


Figure 4.2 Total emitted dose, as % nominal dose, of salbutamol sulphate from the Accuhaler at different inhalation flows (10-60) $Lmin^{-1}$

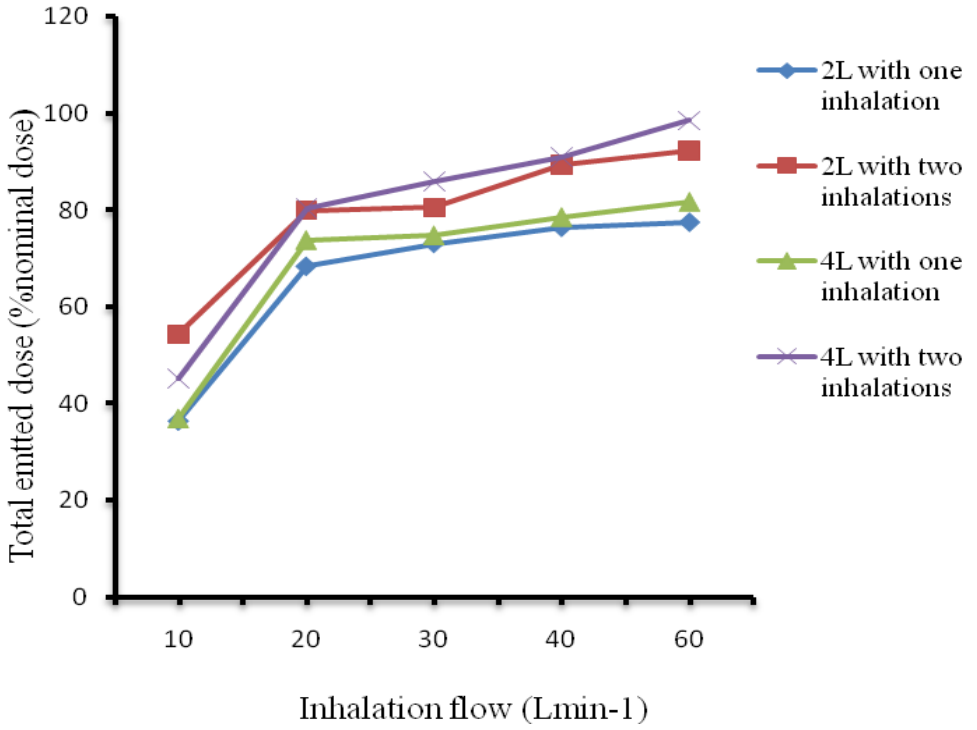


Figure 4.3 Total emitted dose, as % nominal dose, of salbutamol sulphate from the Easyhaler at different inhalation flows (10-60) $Lmin^{-1}$

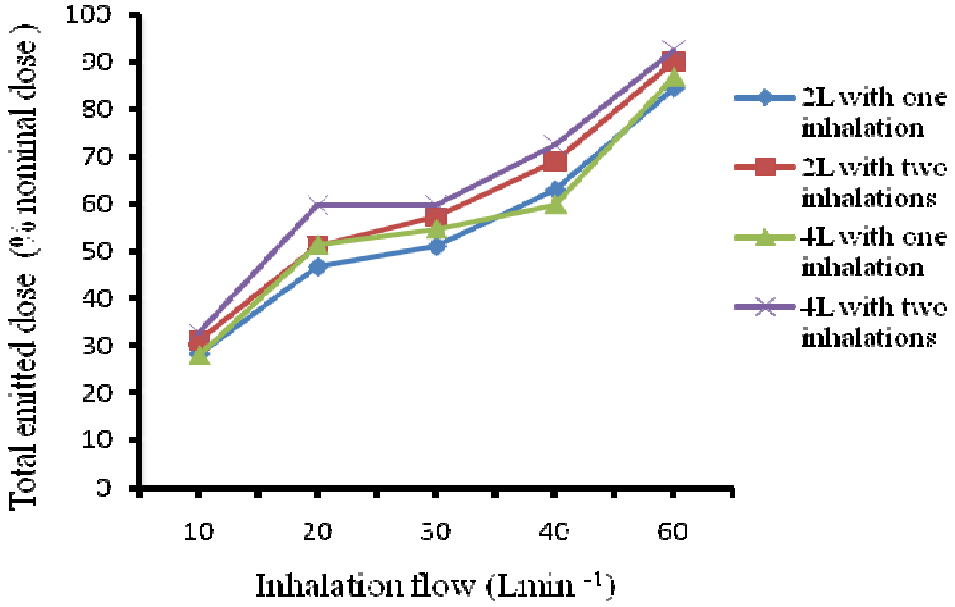


Figure 4.4 Total emitted dose, as % nominal dose, of salbutamol sulphate from the Clickhaler at different inhalation flows (10-60) $Lmin^{-1}$

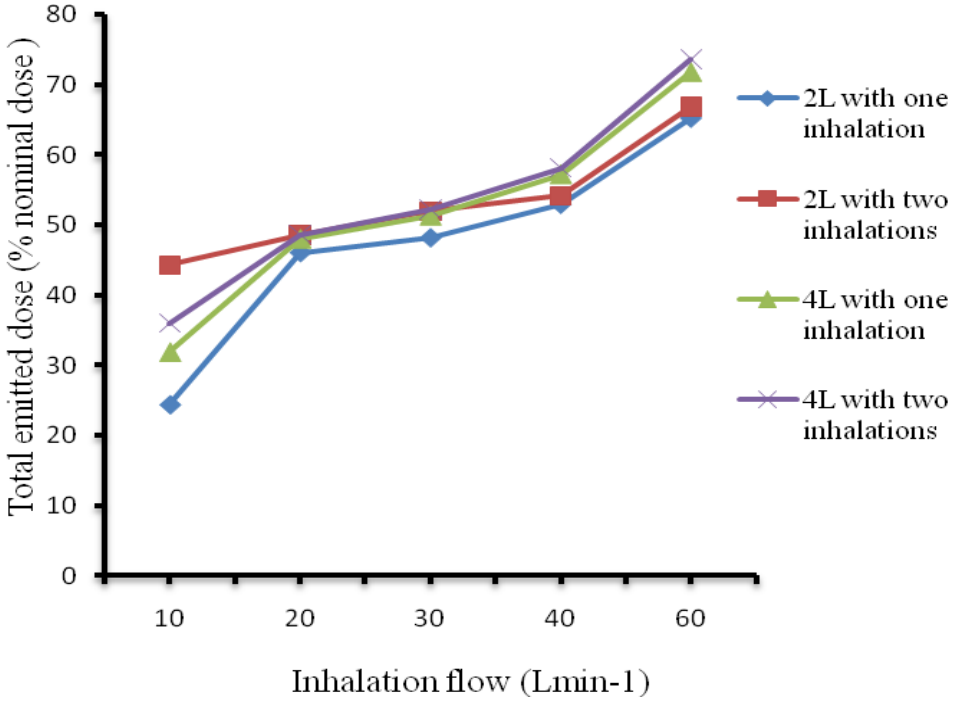


Figure 4.5 Total emitted dose, as % nominal dose, of terbutaline sulphate from the Turbuhaler at different inhalation flows (10-60) $Lmin^{-1}$

Table 4.11 Statistical comparison of the emitted dose (% nominal dose) from the four different dry powder inhalers at varying inhalation flows (10-60) $Lmin^{-1}$ using a 2L inhaled volume

Inhalation flow	**Mean difference (95 % confidence interval)**			
($Lmin^{-1}$)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10vs20	-2.66 (-10.08, 4.74)	-31.89(-37.45, -26.32)	-18.47*(-22.80, -14.13)	-21.67*(-30.01, -13.32)
10vs30	-17.75***(-22.87, -12.63)	-36.62**(-41.89, -31.35)	-22.81***(-30.53, -15.09)	-23.77*(-28.89, -18.65)
10vs40	-22.63***(-30.72, -14.55)	39.95***(-44.52, -35.37)	-34.76***(-40.76, -28.76)	-28.54**(-31.72, -25.37)
10vs60	-33.79***(-37.84, -29.74)	-40.99***(-49.15, -32.83)	-56.36***(-62.45, -50.27)	-40.79***(-44.41, -37.18)
20vs30	-15.08*** (-22.61, -7.55)	-4.70(-11.35, 1.95)	-4.34(-12.06, 3.37)	-2.10(-8.38, 4.17)
20vs40	-18.55***(-26.35, -10.75)	-7.96*(-14.81, -1.30)	-16.29***(-24.01, -8.57)	-6.87*(-13.15, -0.59)
20vs60	-31.12*** (-38.65, -23.59)	-9.00**(-15.66, -2.35)	-37.89***(-45.61, -30.17)	-9.28**(-15.56, -3.00)
30vs40	-3.46 (-11.27, 4.33)	-3.26(-9.91, 3.39)	-11.95*(-19.675, -4.23)	-4.77(-11.05, 1.50)
30vs60	-16.03(-23.56, -8.50)	-4.30(-10.95, 2.35)	-33.55***(-41.27, -25.83)	-7.18*(-13.46, -0.90)
40vs60	-12.56** (-20.36, -4.76)	-1.04(-7.69, 5.61)	-21.60***(-29.32, -13.87)	-2.41(-8.69, 3.86)

*The mean difference is significant *(P<0.05); ** (P<0.01); *** (P<0.001)

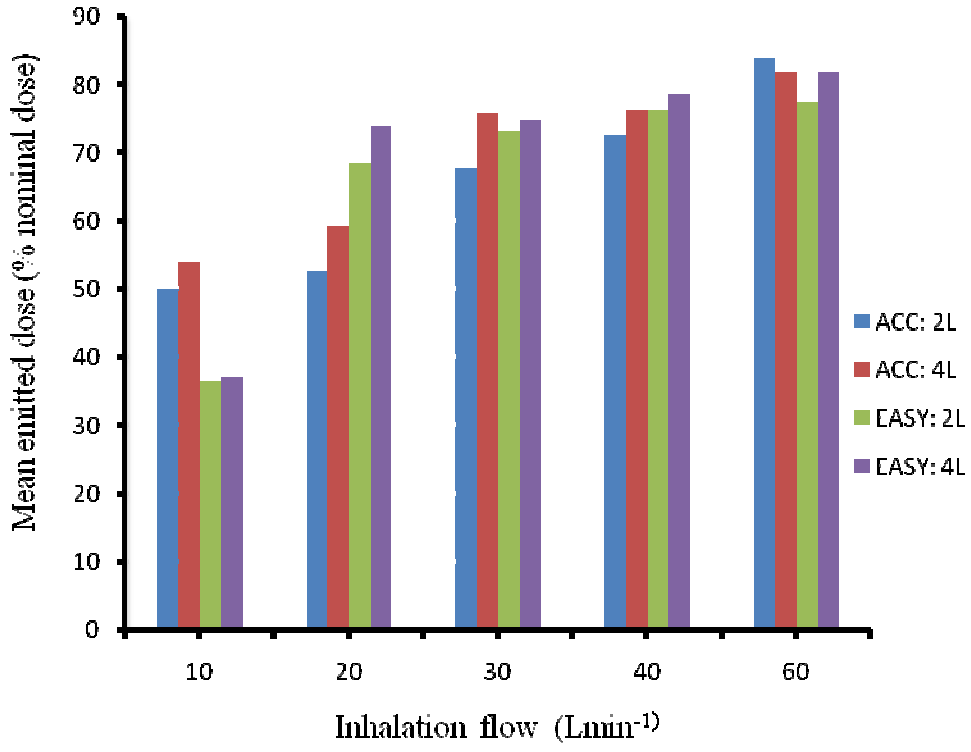
Table 4.12 Statistical comparison of the emitted dose (% nominal dose) from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ using a 4L inhaled volume

Inhalation flow	**Mean difference (95% confidence interval)**			
($Lmin^{-1}$)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10vs20	-5.23(-12.40, 1.94)	-36.79*(-45.26, -28.32)	-23.38*(-30.65, -16.10)	-16.09*(-22.42, -9.76)
10vs30	-21.73**(-27.84, -15.63)	-37.77*(-45.98, -29.56)	-26.79**(-31.87, -21.71)	-19.38***(-24.69, -14.08)
10vs40	22.3***(-29.05, -15.63)	-41.53**(-45.28, -37.77)	-39.21***(-46.85, -31.68)	-25.27***(-29.72, -20.81)
10vs60	-27.69***(-34.90, -20.48)	-44.68**(-48.52, -40.85)	-58.96*(-65.04,-52.89)	-39.78***(-45.36,-34.21)
20vs30	-16.50***(-23.49, -9.50)	-0.97 (-8.59, 6.63)	-3.41(-10.84, 4.02)	-3.29(-8.08, 1.49)
20vs40	-17.06***(-24.07, -10.07)	-4.73(-12.35, 2.87)	-15.89***(-23.32, -8.45)	-9.17***(-13.97, -4.38)
20vs60	-22.46***(-29.45, -15.46)	-7.89*(-15.51, -0.27)	-35.58***(-43.02, -28.14)	-23.69***(-28.49, -18.90)
30vs40	-0.57 (-7.56, 6.42)	-3.75(-11.37, 3.85)	-12.48**(-19.91, -5.04)	-5.88*(-10.67, -1.08)
30vs60	-5.96 (-12.95, 1.04)	-6.91(-14.53, 0.70)	-32.17***(-39.61, -24.73)	-20.40***(-25.19, -15.60)
40vs60	-5.39 (-12.38 , 1.60)	-3.15(-10.77, 4.46)	-19.69***(-27.13, -12.25)	-14.51***(-19.31, -9.72)

*The mean difference is significant *(P<0.05); ** (P<0.01); *** (P<0.001)

Figure 4.6 describes the effect of a 2L inhaled volume and a 4L inhaled volume on the emitted dose at each inhalation flow with one inhalation per metered dose. Table 4.12 presents a statistical comparison of these results.

Figures 4.7 and 4.8 describe the difference in the emitted dose from the Accuhaler, Easyhaler, Clickhaler and Turbuhaler between one inhalation and two inhalations at each inhalation flow for a 2L and a 4L inhaled volume. Tables 4.13 and 4.14 show the statistical comparison of the emitted dose (% nominal dose) from the four dry powder inhalers between one and two inhalations per metered dose at each inhalation flow using 2L and 4Linhaled volumes.



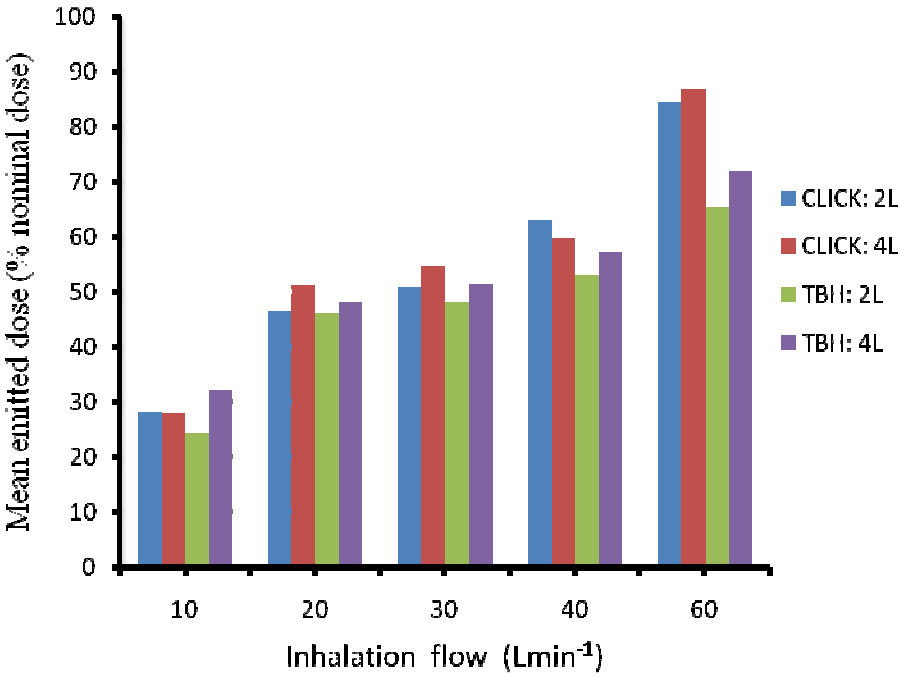


Figure 4.6 Mean emitted dose, as % nominal dose, from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per metered dose for 2L and 4L inhalation volumes

Table 4.13 Statistical comparison of the emitted dose (% nominal dose) from the four different dry powder inhalers between 2L and 4L inhaled volumes each inhalation flow

Inhalation flow ($Lmin^{-1}$)	**Mean difference (95% confidence interval) (2L vs. 4L)**			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10	-4.02(-9.54, 1.48)	-0.58(-4.72, 3.55)	0.28(-4.03, 4.60)	-7.55(-13.58, -1.51)
20	-6.59(-17.04, 3.86)	-5.48(-14.67, 3.70)	-4.62(-9.96, 0.71)	-1.97(-8.87, 4.92)
30	-8.00*(-13.21, -2.80)	-1.73(-11.04, 7.58)	-3.69(-11.92, 4.53)	-3.16(-10.11, 3.78)
40	-3.69(-10.37, 2.99)	-2.16(-7.10,2.77)	-4.22(-11.90, 3.46)	4.27(-8.36, -0.19)
60	2.06*(0.23, 3.90)	-4.27(-12.21, 3.66)	-2.31(-10.09, 5.45)	6.34*(-10.27, -2.80)

*The mean difference is significant *(P<0.05); ** (P<0.01); *** (P<0.001)

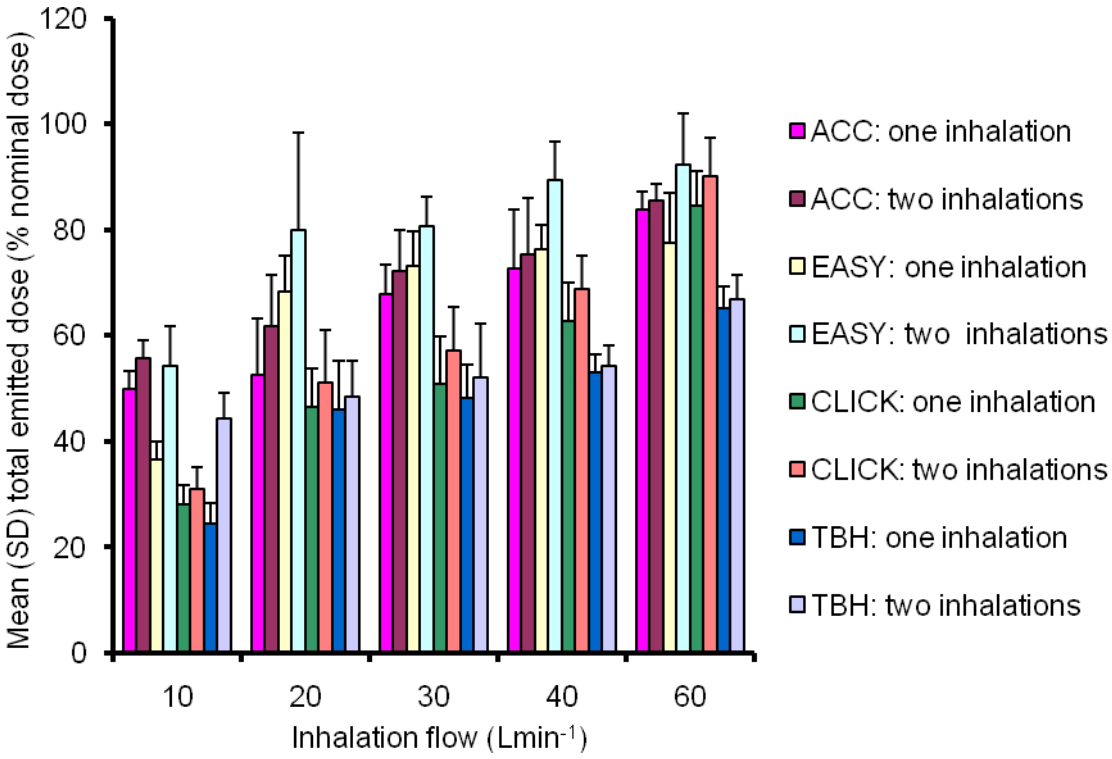


Figure 4.7 Mean (SD**)** total emitted dose, as % nominal dose, from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ following one and two inhalations per metered dose for a 2L inhaled volume

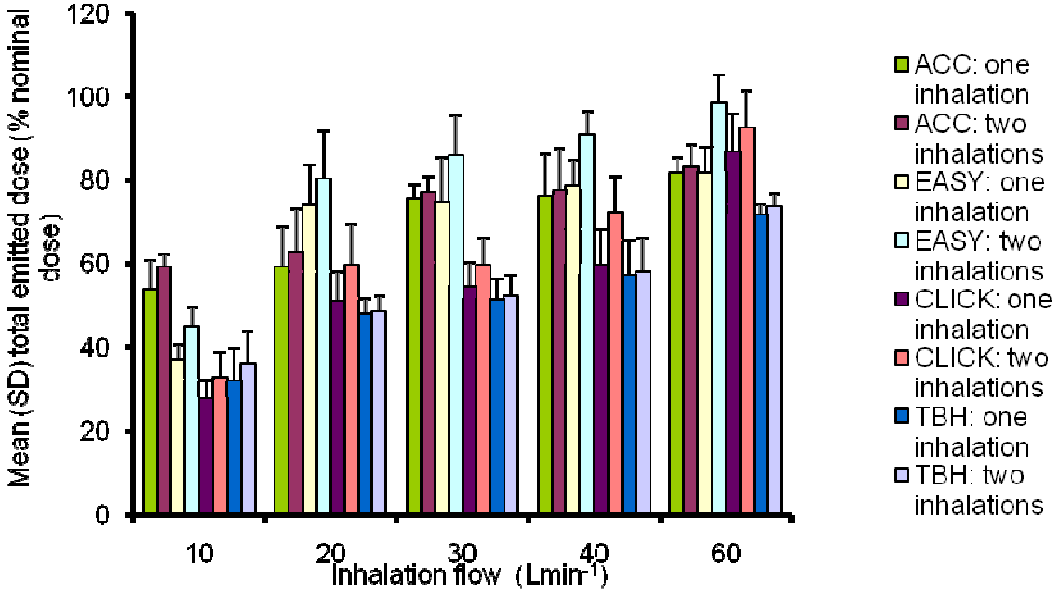


Figure 4.8 Mean (SD) total emitted dose, as % nominal dose, from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ following one and two inhalations per metered dose for a 4L inhaled volume

Table 4.14 Statistical comparison of the emitted dose (% nominal dose) from the four different dry powder inhalers between one and two inhalations per metered dose at each inhalation flow using 2L inhaled volume

Inhalation flow ($Lmin^{-1}$)	**Mean difference (95% confidence interval) (one vs. two inhalations)**			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10	-6.24**(-10.93, -1.54)	-17***(-24.53, -11.22)	-2.92(-7.08, 1.22)	19.85*(-24.38, -15.33)
20	-9.24**(-14.22, -4.26)	-6.59***(-7.78, -5.40)	-4.57**(-7.54, -1.59)	-5.87***(-7.35, -4.39)
30	-4.55*(-8.26, -0.85)	-7.48***(-8.45, -6.51)	0.00 (0.00, 0.00)	-0.33 (-0.68, 0.02)
40	-2.78 (-5.65, 0.089)	-12.96***(-16.60, -9.32)	-6.00***(-6.97, -5.04)	-1.20 (-2.90, 0.49)
60	-1.84 (-3.107, -0.59)	-14.91***(-16.47, -13.36)	-5.57***(-6.45, -4.70)	-1.68*(-3.30, -0.06)

*The mean difference is significant *(P<0.05); ** (P<0.01); *** (P<0.001)

Table 4.15 Statistical comparison of the emitted dose (% nominal dose) from the four different dry powder inhalers between one and two inhalations per metered dose at each inhalation flow using 4L inhaled volume

Inhalation flow ($Lmin^{-1}$)	**Mean difference (95 % confidence interval) (one vs. two inhalations)**			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10	-5.20**(-10.80, 0.40)	-8.07***(-11.03, -5.12)	4.88**(-8.35, -1.42)	-4.04(-12.45, 4.35)
20	-3.58** (-5.65, -1.51)	-6.57***(-8.51, -4.62)	-8.53**(-13.19, -3.87)	-0.58 (-1.30, 0.13)
30	-14.13** (-23.29, -4.98)	-11.20***(-14.04, -8.37)	-5.15***(-6.31, -3.99)	-0.87**(-1.32, -0.42)
40	-1.29 (-3.17, 0.58)	-12.47***(-13.36, -11.58)	-5.28***(-5.77, -4.79)	-0.90***(-1.32, -0.48)
60	-1.39 (-3.58, 0.80)	-16.83***(-17.68, -15.98)	-5.79***(-6.85, -4.72)	-1.82***(-2.65,-1.00)

*The mean difference is significant *(P<0.05); ** (P<0.01); *** (P<0.001)

4.4 Discussion

In this chapter the total emitted dose from the Accuhaler®, the Turbuhaler, ® the Clickhaler® and the Easyhaler® DPIs as a function of inhalation flow, inhalation volume and the number of inhalations per metered dose has been evaluated. Each of these inhalers differs in device-design and formulation.

The Accuhaler® contains multiple unit doses that are each dose factory dispensed in a sealed blister on a long strip. In contrast, the Turbuhaler®, Clickhaler® and Easyhaler® are the reservoir-type of multidose inhalers. In this type of inhalers, the powder formulation is stored in a reservoir from which single doses are volumetrically measured and dispensed using a special dose metering unit. The powder formulations in these devices also differ. The Accuhaler, Clickhaler, and Easyhaler-all contain salbutamol formulated from the admixture of pure drug and lactose carrier, while the Turbuhaler contains terbutaline formulated from pure spherical pellets of the pure drug. Thus, it is possible that the effects of the inspiratory parameters on the total emitted dose may differ.

Several in-vitro studies have shown that the emitted dose from different DPIs varied with a change in the inhalation flow to a varying extent (de Boer et al, 1996; Malton et al, 1995; Palander et al, 2000; Tarsin et al, 2004). Tarsin et al. (2004) have measured the in-vitro dosage emission and the fine particle dose (FPD) from 100/6 and 200/6 Symbicort Turbuhalers (budesonide and formoterol) at different flows (30, 60 and 90 $Lmin^{-1}$). The data showed that the amounts of budesonide and formoterol emitted from the Symbicort 100/6 and Symbicort 200/6 inhalers were affected by the increased inhalation flow. Similarly Palander et al, 2000 using the Accuhaler, Easyhaler and Turbuhaler showed that the mean (SD) emitted dose (% nominal dose) of salbutamol increased from 76 (12), 95(4.8), and 72(22) at inhalation flow 30 $Lmin^{-1}$ to 92(9), 95 and 88(19) at inhalation flow of 60 $Lmin^{-1}$ respectively. A study by de Boer et al (1996) showed that the emitted dose from three different DPIs (Spinhaler, Turbuhaler and Diskhaler) significantly increased

with an increase in inhalation flow. The Diskhaler was the most sensitive to inhalation flow, emitting <30% of the claimed label dose at 20 $Lmin^{-1}$. The results identified in this chapter indicated that the total emitted dose (% nominal dose) from the Accuhaler, Easyhaler®, Clickhaler® and Turbuhaler® increased to varying extent with respect to the inhalation flow within the flow range of 10 to 60 $Lmin^{-1}$ when tested using the same inhalation volume with one or two inhalations for each dose as shown in Figures 4.2 to 4.5. Furthermore, the data presented in Table 4.9 show that when using a 4L inhalation volume the mean emitted dose (% nominal dose) from the Accuhaler® and the Easyhaler® increased from 75.73 and 74.81 at an inhalation flow of 30 $Lmin^{-1}$ to 81.69 and 81.72 at an inhalation flow of 60 $Lmin^{-1}$ respectively. Similarly, the mean emitted dose (% nominal dose) from the Clickhaler® and the Turbuhaler® increased from 54.59 to 86.76 and 51.35 to 71.75 respectively. The increase in the total emitted dose from the Clickhaler and the Turbuhaler® is steeper than that from the Accuhaler® and the Easyhaler® (Figures 4.2 to 4.5, demonstrating higher sensitivity to the inhalation flow at the range of 30 to 60 $Lmin^{-1}$. Statistical comparison of the emitted dose from the studied inhalers between inhalation flows (Tables 4.10 and 4.11) showed that although the emitted dose increased upon increasing the inhalation flow from 10 to 60 $Lmin^{-1}$, generally there was no statistically significant difference with the Accuhaler and the Easyhaler at inhalation flow range of 30 to 60 $Lmin^{-1}$. This is true when inhaled volumes of 2L and 4L were used with the Easyhaler. However, flow dependent dose emission was significant at the inhalation flow range of 10 to 30 $Lmin^{-1}$ when tested using inhaled volumes of 2L and 4L with the Accuhaler® ($p>0.001$) and the Easyhaler® ($p>0.05$). In contrast, the Clickhaler® and the Turbuhaler® showed significant ($p>0.001$) flow dependent dose emission property throughout the inhalation flow range of 10 to 60$Lmin^{-1}$when tested using inhaled volumes of 2L and 4L. De Boer et al (1996) have attributed these differences in behaviour to differences in device-design and the type of powder formulation. The results of this study

are consistent with the previous in-vitro study by Palander et al (2000) which highlights that the Accuhaler and Easyhaler are less affected by changes in inhalation flows than the Turbuhaler.

The effect of inhalation volume (which translates to inspiration time) on the emitted dose from the Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler® is presented in Figures 4.6. Statistical comparison of the emitted dose from these inhalers between a 2L inhaled volume and a 4L inhaled volume at each inhalation flow is presented in Table 4.12. Overall, there was insignificant difference in the emitted dose between 2L and 4L inhalation volumes across the inhalation flow range of 10 to 60Lmin^{-1}. The Accuhaler contains multiple doses with each dose factory-dispensed dose in a blister on a strip inside the device, while the Turbuhaler®, Clickhaler® and Easyhaler® are the reservoir-type of multidose inhalers. This finding is consistent with a previous study by De Boer et al (1996) who found that the effect of inspiration time (inhalation volume) on the dose emission from the Turbuhaler (reservoir) and the Diskhaler® (blister) over the same flow range was negligible for all four inspiration times (0.5, 1.5, 3.0 and 6 s) used. Although it was expected that a higher emitted dose may be obtained with a larger inhaled volume (because of the greater energy input), no significant difference was observed in this study. This may be attributed to particles being lifted from dose metering cup/strip and carried ex-mouthpiece of an inhaler early in the simulated inhalation. Previously Everard et al (1997) have reported that dose emission from DPIs formulated with either a reservoir or blister occurs immediately at the start of the inhalation. Thus, for these types of DPIs energy impacted on the powder to overcome attractive forces between particles, or the particle and carrier, by a 2L volume may not be significantly different from that provided by a 4L inhaled volume. Hence, the effect of inhalation volume on the emitted dose is negligible highlighting the acceleration effect reported by Everard et al (1997).

The effect of the number of inhalations on the emitted dose from dose system of the Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler® at different inhalation flows (10 to 60 $Lmin^{-1}$) using the same inhalation volume is shown in Figures 4.7 and 4.8 (for 2L and 4 L inhalation volumes). The emitted dose from the four inhalers following two inhalations per metered dose is greater than that for one inhalation. These differences in the emitted dose between one and two inhalations were different for each type of inhaler studied. The differences were confirmed by statistical comparison of the emitted dose between one and two inhalations at different inhalation flows (10 to 60 $Lmin^{-1}$) using the same inhalation volume (Tables 4.13 and 4.14). The emitted dose from the Accuhaler was significantly ($p>0.001$) greater with two inhalations than one inhalation for each dose at inhalation flows below 30 $Lmin^{-1}$. However, this trend was variable with the Turbuhaler. At the flow range of 30 to 60 $Lmin^{-1}$ using a 4L inhaled volume, the emitted dose was significantly greater with two inhalations than one inhalation while for a 2L inhaled volume this trend occurred at the flow range of 10 to 30$Lmin^{-1}$. On the other hand, the emitted dose from the Easyhaler® and the Clickhaler® was significantly ($p<0.001$) greater with two inhalations than one inhalation across the inhalation flow of 10 to 60 $Lmin^{-1.}$

4.5 Conclusion

In conclusion, this study highlights that the Accuhaler, the Easyhaler, the Clickhaler, and the Turbuhaler all showed flow-dependent dose emission to a varying extent due different device-design and powder formulation.

Furthermore, the Accuhaler®, Easyhaler®, Clickhaler®, and the Turbuhaler® showed insignificant difference in the total emitted dose between a 2L inhalation volume and a 4L inhalation volume at different inhalation flows. This study shows that inhalation volume is not critical for these inhalers above a volume of 2L. Patients with either asthma or COPD have an average inhalation volume of 2L when they inhaled through a DPI (Hawskworth, et al. 2000). Since multidose inhaled products are for the use of these patients then it is

important to perform the in-vitro assessment of the inhaled products using the inhalation volume of 2L rather than 4L the recommended by the compendial method consistent with the US Food and Drug Administration (FDA 1998) guidelines.

The Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler® generally showed significantly greater total emitted dose after two inhalations than one inhalation per metered dose at inhalation flow less than 30 $Lmin^{-1}$. This implies that patients with low inspiratory flows through these DPIs may benefit more from inhaling twice for each metered dose. At present the patient's information leaflets for the studied inhalers instruct one inhalation per metered dose `as hard and fast as they can`.

The fine particle dose (the portion of the inhaler output containing particles in the size range $\leq 5\mu m$), and its aerodynamic particle size distribution determines the lung deposition and ultimately the clinical effects. Thus, the findings about the effects of the inhalation flow, the inhalation volume and the number of inhalations on the total emitted dose identified in this chapter need to be further evaluated by the measurement of aerodynamic dose emission characteristics of the Accuhaler, Easyhaler, Clickhaler and the Turbuhaler using the same inhalation criteria used in this study.

Chapter 5

5 In-vitro determination of aerodynamic dose emission characteristics of dry powder inhalers at different inhalation flows using a mixing inlet

5.1 Introduction

The fine particle dose (FPD) which is defined as that component of the emitted drug with an aerodynamic particle diameter of <5μm, comprises particles in the `respirable range` that have the greatest potential for lung deposition (Chrystyn, 2006). A study by Olsson et al. (1996) suggests that there is a correlation between lung deposition of salbutamol and the in-vitro fine particle dose obtained using a cascade Impactor. Other studies (Engel et al 1992; Hirsch et al, 1997) have shown that the fine particle dose is related to lung deposition and ultimately the clinical effect, whilst in-vitro studies have reported (Palander, et al, 2000; Ross and Schultz 1996) that this dose is dependent on the speed of the inhalation used.

The fine particle dose together with the aerodynamic particle size distribution characteristics of DPIs can be measured in-vitro using the Andersen Cascade Impactor (ACI). The methodology is described in the United State Pharmacopoeia (USP 2009), European Pharmacopoeia (EP 2007) and British Pharmacopoeia (BP 2008). Traditionally, the ACI has been designed for operation at a flow rate of 28.3 $Lmin^{-1}$. Use of different flows will alter the cut-off diameter of each stage of the Impactor (Van Oort, 1995). To overcome this problem, modifications to the stages of the ACI have recently been introduced by replacing some stages. However, the stage replacements restrict the determination of dose emission characteristics to 28.3, 60 and 90 $Lmin^{-1}$ but the inhalation profiles generated by patients through different inhalers do not result in fixed steady-state flows. It is, therefore, important to determine dose emission characteristics from an inhaler at a variety of flows to include those outside the fixed standard flows and thus be more consistent with the patient's normal use of inhale products. A mixing inlet has been specially designed to provide a fixed flow (as for example 60 $Lmin^{-1}$) through the impactor,

whilst permitting variable flows (below 60 $Lmin^{-1}$) through the inhaler (Copley, 2007). Therefore no calculations are required to adjust the cut-off diameter of each stage.

The studies in this chapter have been designed to identify the effect and influence of inhalation flow, inhalation volume and the number of inhalations for each dose on the fine particle dose and its particle size distribution characteristics. Four different DPIs namely the Accuhaler®, the Clickhaler®, the Easyhaler® and the Turbuhaler® have been used to identify the minimum criterion of the inhalation manoeuvre to deliver a dose into the lungs of patients.

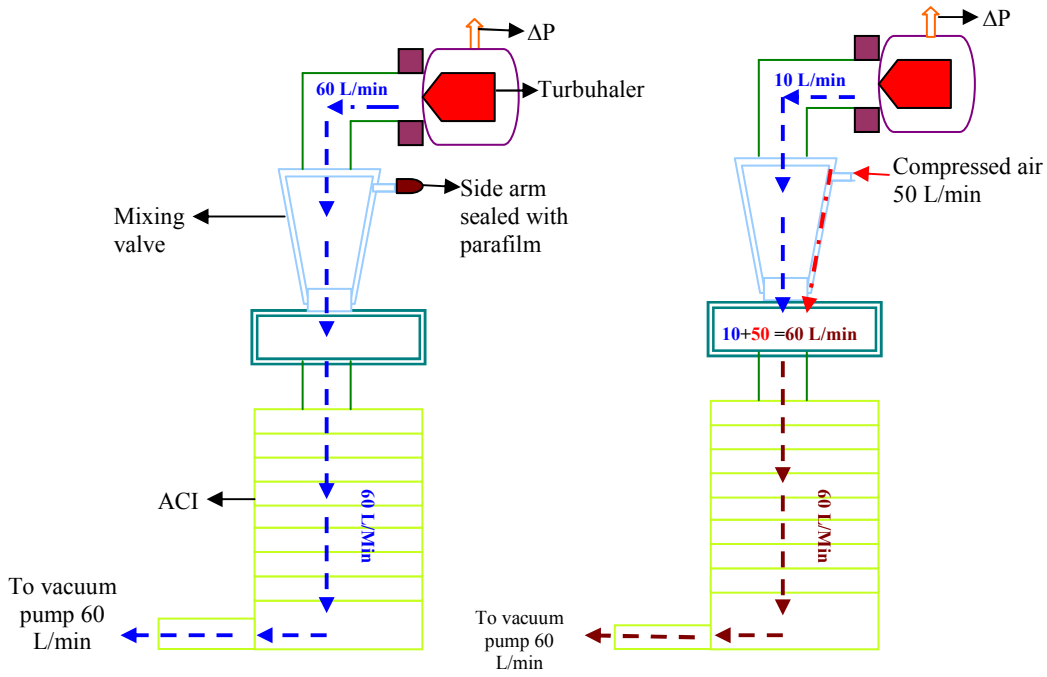


Figure 5.1 (a) The ACI assembled with the mixing inlet side arm sealed (b) Inhalation flow set up with compressed air passed into the ACI via the mixing inlet valve

5.2 Method

5.2.1 Instruments and inhaler devices

Andersen MKII Cascade Impactor	Copley Scientific Ltd, UK
Critical flow controller model TPK	Copley Scientific Ltd, UK
Mixing inlet	Copley Scientific Ltd, UK
GF 50 filter	Copley Scientific Ltd, UK
GAST pump	Brook Crompton, UK
An electronic digital flow meter	MKS Instrument, USA
Parafilm M laboratory film	Pechiney Plastic Packaging, USA
Silicone fluid spray	Releasil B silicone spray, Dow Corning Limited, Barry, Glamorgan, UK

Inhaler devices: Ventolin®Accuhaler® [ACC] containing salbutamol sulphate 200 µg per dose (GlaxoSmithKline); Clickhaler® [CLICK] containing salbutamol sulphate 114 µg per dose; Easyhaler® [EASY] containing salbutamol sulphate 200 µg per dose (Orion Pharma, Finland); and

Bricanyl® Turbuhaler® [TBH] containing terbutaline sulphate 500 µg per dose, (Astra Zeneca, UK).

5.2.2 Procedure

The ACI and its accessories (preseparator and induction port) were washed with methanol/water and dried at a room temperature. The collection plates were sprayed with silicone and allowed to dry prior to use. The ACI stages were assembled with 10ml of bamethane in distilled water (internal standard) in the preseparator and a GF50 (Copley

Scientific Ltd, UK) filter located in the final stage. The USP (2009) recommends the use of the pre-separator containing about 10ml of washing solution for DPIs to entrain large particles usually greater than 10μm. The Impactor was initially set up with the arm of the mixing inlet closed to set the airflow at 60 $Lmin^{-1}$ so stages 0 and 7 were replaced by -1 and -0. The inhalation time (discharge time) was set as described in chapter 4. Figure 5.1 shows that the inhaler, enclosed in an airtight chamber, was attached to the induction port of the ACI. The inhaler was placed inside the chamber to enable the pressure drop to be monitored. The ACI was then connected to a vacuum pump via the critical flow controller (model TPK). The flow control valve was adjusted until a flow of 60 L/min was achieved (also sonic flow achieved $P2/P3 \leq 0.5$). Then the side arm of the mixing inlet was opened and supplementary compressed air allowed through until a desired flow (say 10L/min) through the inhaler device was achieved, whilst maintaining the fixed flow of 60 $Lmin^{-1}$ through the impactor as shown in Figure 5.1. The inhaler was detached from the induction port and loaded before it was attached again to the port for discharge of the drug into the impactor by activating solenoid valve. Following the discharge, the inhaler device was flushed to waste at flow of 90 L min^{-1} to remove any remaining powder before proceeding to the next determination. The induction port, mixing inlet together with the preseparator, stage-plates and the filter were separately rinsed with standard aqueous bamethane solution (8000 μgL^{-1}) according the washing volumes shown in Table 5.1. The amounts of active drug collected on the filter and deposited on the ACI stages were determined by the high liquid performance chromatography (HPLC) method described in chapter 3.This procedure was similarly carried out for each determination using two inhalations per metered dose, except that the TPK timer controlling the solenoid valve was activated twice to obtain two separate discharges for each loaded dose into the impactor at each inhalation flow.

Table 5.1 Washing volume for each stage of the ACI

Stage	Volume of washing (ml)				
	10 L min^{-1}	20 L min^{-1}	30 L min^{-1}	40 L min^{-1}	60 L min^{-1}
Induction port	25	25	50	50	50
Preseparator	25	25	50	50	50
-1	10	10	10	10	10
-0	10	10	10	10	25
1	10	10	25	25	50
2	10	10	25	25	25
3	10	10	25	25	25
4	10	10	10	10	25
5	5	5	10	10	25
6	5	5	10	10	10
Filter	5	5	10	10	10

The aerodynamic dose emission characteristic of terbutaline sulphate from Bricanyl® Turbuhaler® and salbutamol sulphate from the Accuhaler®, Clickhaler® and Easyhaler® at inhalation flows of 10, 20, 30, 40, and 60 $Lmin^{-1}$ following one and two inhalations per dose for a 2L inhaled volume and a 4L inhaled volume was determined.

A total of five separate dose determinations (n=5); two at the beginning, one in the middle and two drawn at the end of the lifetime of each type of inhaler, were performed and analysed for each type of inhaler. The dose numbers used was randomised.

5.2.3 Data analysis

A plot of the logarithm of the percentage less than a stated size on a probability scale against the logarithm of the effective cut-off diameter of the stage was constructed (United States Pharmacopeia 2005). Copley Inhaler Testing Data Analysis Software (CITDAS) was used to identify the aerodynamic characteristics of the emitted dose. The fine particle dose (FPD) was the amount with particles that correspond to a size less than 5μm. The fine particle fraction % (FPF) was the FPD expressed as a percentage of the total amount deposited into the throat and stages of the cascade impactor (this is the dose exiting the mouthpiece) as well as expressed as a percentage of the nominal dose (label claim). The

mass median aerodynamic diameter (MMAD) was the diameter corresponding to 50% undersize. The geometric standard deviation (GSD) was the square root for the size corresponding to 84.13% less than the stated size divided by the square root of the size for 15.87% (United States Pharmacopeia 2005). Since the label claims of the studied inhalers differ, the total emitted dose (TED) and the fine particle dose (FPD) from each inhaler were respectively expressed as percentage nominal dose (label claim).

5.2.4 Statistical analysis

SPSS version 15.0 software (SPSS Inc., Chicago, USA) was used for the statistical analysis. A two-way analysis of variance (ANOVA) with the application of the General Linear Model Univariate was used to determine any significant differences in the fine particle dose from the four different inhalers at different flows. Also the statistical comparisons of the fine particle dose between two different inhalation volumes as well as between one and two inhalations for each metered dose at the same flows were made. The mean difference (95% confidence interval) was calculated and a probability value of ($p<0.05$) was considered being significant.

5.3 Results

The mean (SD) amount of drug deposited on each stage of the ACI, total emitted dose, fine particle dose, and the mass median aerodynamic diameter (MMAD) of drug particles from each dry powder inhalers (DPIs) at varying inhalation flows (10-60) $Lmin^{-1}$ following one and two inhalations using 2L and 4L inhaled volume respectively are shown in Tables 5.2 to 5.17. Figures 5.2 to 5.17 describe the amount of drug deposited on each stage of the ACI.

Table 5.2 Mean (SD) Dose emission of salbutamol from the Accuhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow ($Lmin^{-1}$)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	66.07(2.8)	41.89(12.3)	45.30(4.2)	41.92(2.2)	37.09(1.7)
Preseparator	23.54(1.5)	35.77(6.1)	63.02(5.3)	70.61(5.7)	77.91(4.7)
-1	1.11(0.9)	1.04(0.3)	2.65(0.4)	1.97(0.3)	2.30(1.2)
0	1.11(0.6)	1.22(0.2)	2.62(0.8)	2.45(0.8)	2.74(1.53
1	2.27(1.4)	2.74(0.2)	5.73(0.5)	5.90(0.5)	5.22(0.4)
2	4.08(1.3)	5.44(0.5)	11.27(0.8)	11.15(0.8)	11.41(0.9)
3	10.05(1.0)	15.61(1.1)	27.21(7.7)	29.11(4.3)	30.23(0.6)
4	5.31(1.7)	10.36(3.9)	10.90(2.1)	15.85(0.6)	19.15(2.3)
5	1.87(1.6)	2.44(3.1)	3.39(1.6)	4.29(0.8)	5.40(0.6)
6	0.67(0.2)	0.66(0.6)	1.28(0.5)	2.52(1.3)	1.64(5.5)
Filter	0.69(0.5)	2.59(0.2)	1.60(0.9)	2.38(0.3)	2.18(2.9)
TED (µg)	116.76(7.0)	119.89(0.5)	174.96(15.2)	188.17(7.9)	195.28(2.5)
TED (% nominal dose)	58.38(3.5)	60.12(6.9)	87.86(7.5)	93.03(4.0)	97.64(2.2)
FPD (µg)	8.17(3.2)	23.17(3.5)	47.78(10.2)	61.36(4.7)	71.90(0.5)
FPD (% Emitted dose)	6.86(2.3)	19.02(2.0)	27.17(3.6)	32.51(2.2)	36.82(0.1)
FPD (% nominal dose)	4.08(1.6)	11.59(1.0)	23.89(0.1)	30.68(2.4)	35.95(1.1)
MMAD (µm)	6.4(0.3)	4.3(0.1)	3.9(0.1)	3.1(0)	2.5(0.1)
GSD	1.8(0.3)	1.7(0.1)	1.80(0.1)	1.7(0.1)	1.7(0.1)

Table 5.3 Mean (SD) Dose emission of salbutamol from the Accuhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	55.16(7.6)	44.49(1.4)	41.20(2.0)	44.43(2.7)	47.19(2.3)
Preseparator	26.94(1.0)	33.30(0.8)	67.15(3.1)	68.59(6.0)	68.86(2.4)
-1	1.47(0.4)	1.530.3)	1.49(1.0)	1.36(0.8)	1.31(0.3)
0	1.99(0.4)	1.45(0.2)	2.07(0.4)	2.46(1.3)	2.10(0.1)
1	5.40(0.7)	2.12(0.5)	4.580.5)	5.07(0.7)	4.31(0.1)
2	8.531.1)	5.17(0.5)	10.74(1.1)	11.46(0.9)	15.28(0.3)
3	10.63(0.6)	16.67(0.6)	26.42(0.7)	28.70(0.6)	29.17(0.9)
4	5.34(0.7)	10.07(0.3)	19.43(1.6)	15.39(0.6)	20.77(0.8)
5	1.80(1.0)	3.32(0.5)	5.15(1.8)	5.12(0.5)	7.59(0.9)
6	0.29(0.1)	1.73(0.5)	1.16(0.4)	1.90(0.6)	1.39(0.1)
Filter	0.48(0.2)	1.80(0.4)	2.27(0.8)	1.05(0.4)	1.63(0.1)
TED (µg)	118.04(7.1)	121.65(1.3)	181.67(3.1)	185.52(9.1)	199.49(1.9)
TED (% nominal dose)	59.02(3.5)	60.83(0.7)	90.80(1.5)	92.76(4.6)	99.74(1.0)
FPD (µg)	7.26(1.4)	28.90(0.7)	56.88(2.8)	59.74(1.4)	77.18(1.6)
FPD (% Emitted dose)	6.17(1.2)	23.76(0.5)	31.12(1.1)	32.24(1.1)	38.69(0.5)
FPD (% nominal dose)	3.63(0.7)	14.45(0.3)	28.44(1.4)	29.87(0.7)	38.59(0.8)
MMAD (µm)	7.9(0.2)	4.2(0.1)	3.5(0.1)	3.2(0.1)	2.5(0)
GSD	1.6(0)	1.7(0)	1.7(0.1)	1.6(0.1)	1.6(0)

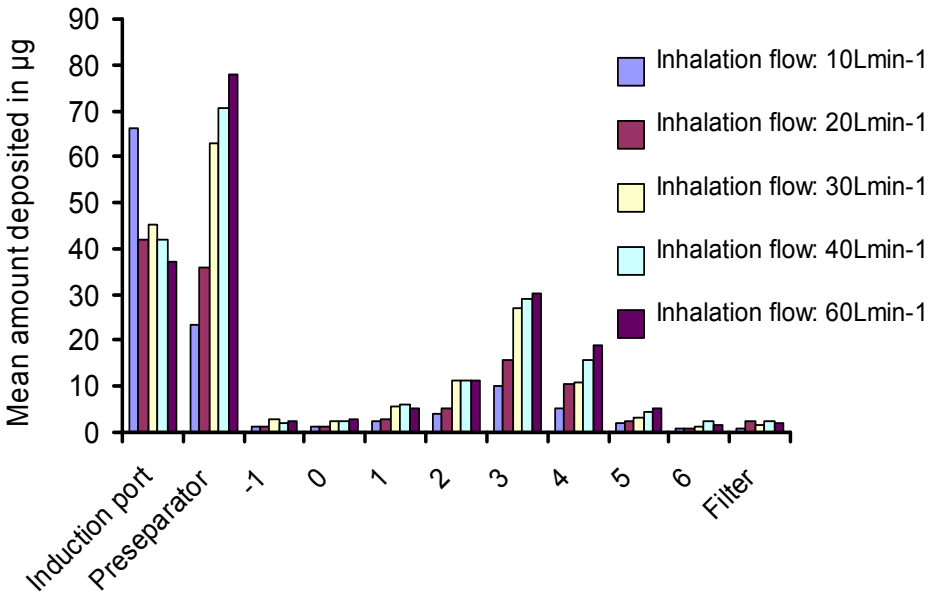


Figure 5.2 Mean amounts (µg) of salbutamol from the Accuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 2L inhaled volume

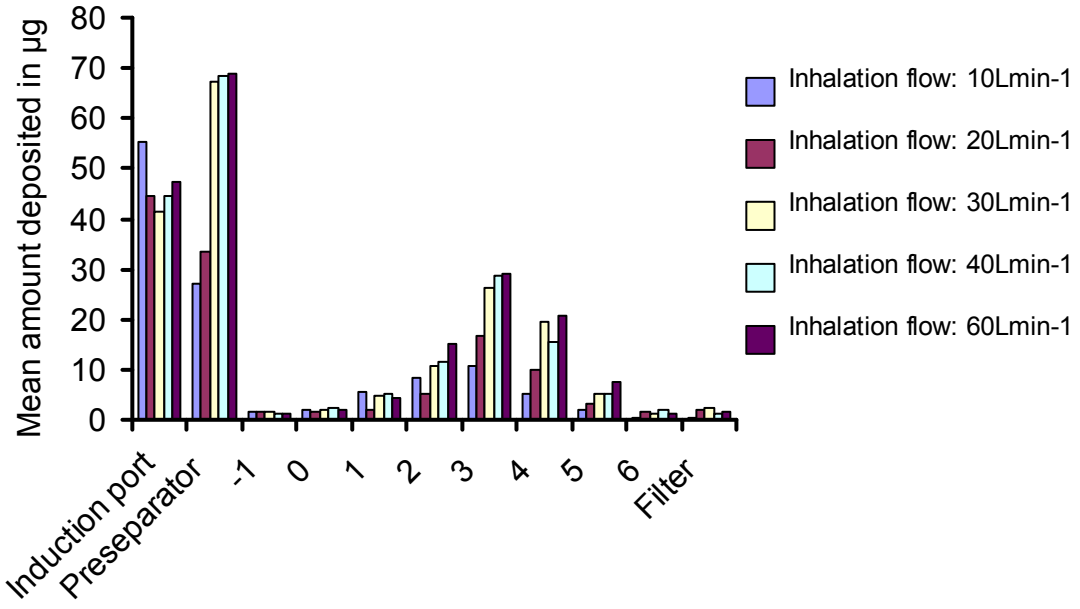


Figure 5.3 Mean amounts (µg) of salbutamol from the Accuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 2L inhaled volume

Table 5.4 Mean (SD) Dose emission of salbutamol from the Accuhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	46.85(3.2)	58.13(7.2)	39.78(8.9)	46.18(2.3)	44.29(1.7)
Preseparator	23.73(5.2)	39.82(6.9)	65.68(5.6)	66.11(2.8)	70.20(2.0)
-1	2.83(0.2)	1.86(1.0)	1.70(0.4)	1.55(0.5)	1.62(0.3)
0	1.58(0.4)	2.74(1.2)	1.88(0.6)	2.41(1.0)	1.95(0.3)
1	2.88(0.9)	5.08(2.5)	4.38(0.7)	5.39(1.4)	4.19(1.3)
2	5.65(0.9)	7.84(1.5)	10.11(1.7)	10.75(2.7)	11.10(1.6)
3	12.14(1.7)	17.19(0.9)	27.72(5.1)	29.45(2.5)	31.20(1.4)
4	9.01(1.2)	9.88(0.3)	18.33(3.7)	18.76(1.2)	20.19(0.8)
5	2.38(0.3)	3.27(0.6)	4.65(1.4)	4.80(0.7)	6.76(1.8)
6	0.91(0.3)	1.95(0.2)	0.90(0.4)	1.74(0.4)	2.05(0.4)
Filter	1.50(0.4)	2.44(0.5)	1.07(0.7)	1.800.9)	1.44(0.6)
TED (µg)	109.47(8.4)	150.22(9.8)	176.22(23.7)	191.00(13.4)	194.99(5.3)
TED (% nominal dose)	54.73(4.2)	75.00(5.1)	88.21(12.0)	95.32(6.7)	97.50(2.7)
FPD (µg)	13.08(1.7)	29.82(3.2)	54.98(9.7)	63.59(5.1)	74.37(3.9)
FPD (% Emitted dose)	11.97(1.5)	19.89(2.0)	31.18(2.9)	33.62(1.4)	38.24(1.6
FPD (% nominal dose)	6.54(0.8)	14.91(1.6)	27.49(4.9)	31.80(2.6)	37.2(1.9)
MMAD (µm)	6.30.2)	4.50.1)	3.5(0.1)	3.1(0.1)	2.4(0)
GSD	2.0(0.1)	1.8(0.1)	1.7(0.1)	1.7(0.1)	1.6(0.1)

Table 5.5 Mean (SD) Dose emission of salbutamol from the Accuhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	45.52(7.4)	62.95(5.4)	50.51(3.0)	42.92(1.1)	40.87(1.6)
Preseparator	26.00(4.6)	43.23(3.0)	66.18(2.0)	70.02(4.2)	68.78(1.5)
-1	2.45(1.4)	1.99(0.6)	2.08(0.5)	1.68(0.7)	1.54(0.3)
0	1.56(0.7)	2.60(0.3)	2.42(0.7)	2.64(0.9)	2.22(0.2)
1	2.97(1.2)	4.68(0.7)	2.28(0.4)	5.54(0.5)	5.49(0.3)
2	5.61(0.5)	9.31(2.0)	9.30(1.0)	12.98(0.7)	15.84(0.4)
3	13.43(1.8)	20.75(2.2)	27.20(0.7)	29.03(0.3)	29.56(0.3)
4	8.59(2.0)	12.69(2.4)	16.81(1.2)	18.56(0.9)	25.47(0.8)
5	2.61(0.8)	4.09(1.0)	5.12(0.6)	4.97(1.5)	9.71(0.4)
6	0.70(0.3)	1.44(0.7)	1.17(0.5)	2.09(0.8)	2.08(0.4)
Filter	1.33(0.5)	1.36(0.5)	1.12(2.3)	1.35(0.3)	1.34(0.2)
TED (µg)	110.76(8.7)	165.10(14.5)	184.19(4.6)	191.76(6.4)	202.90(3.3)
TED (% nominal dose)	55.38(4.4)	82.55(7.3)	92.10(2.3)	95.88(3.2)	101.45(1.7)
FPD (µg)	12.60(2.0)	34.56(5.3)	53.51(1.5)	64.58(1.7)	86.15(0.8)
FPD (% Emitted dose)	11.48(2.3)	19.90(2.7)	81.86(1.2)	33.70(1.1)	42.52(0.6)
FPD (% nominal dose)	6.30(1.0)	17.28(2.6)	26.76(0.7)	32.29(0.8)	43.07(0.4)
MMAD (µm)	6.0(0.4)	4.6(0.2)	3.5(0)	1.7(0.1)	2.4(0)
GSD	1.9(0.2)	1.8(0)	1.62(0.1)	32.29(0.1)	1.7(0)

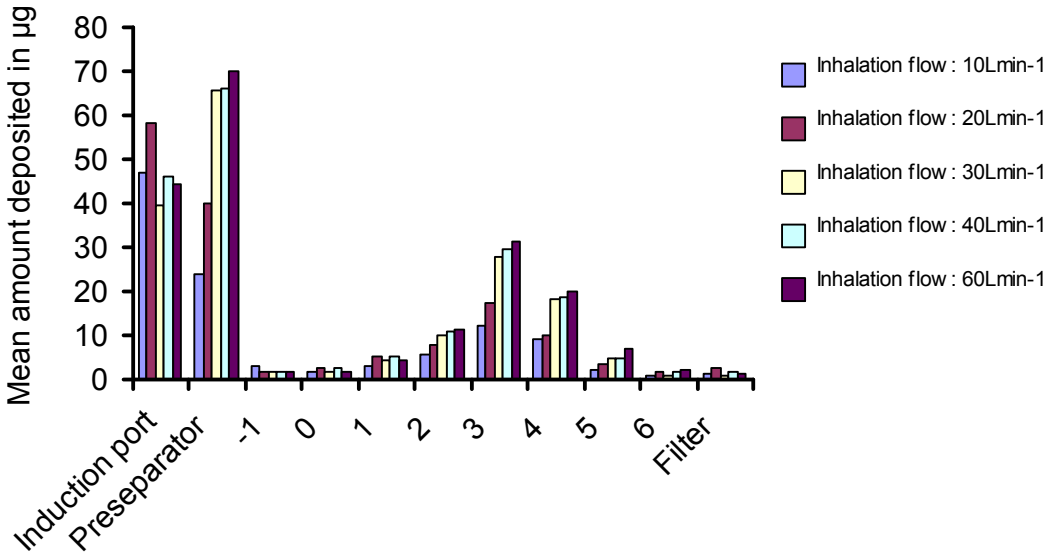


Figure 5.4 Mean amounts (µg) of salbutamol from the Accuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume

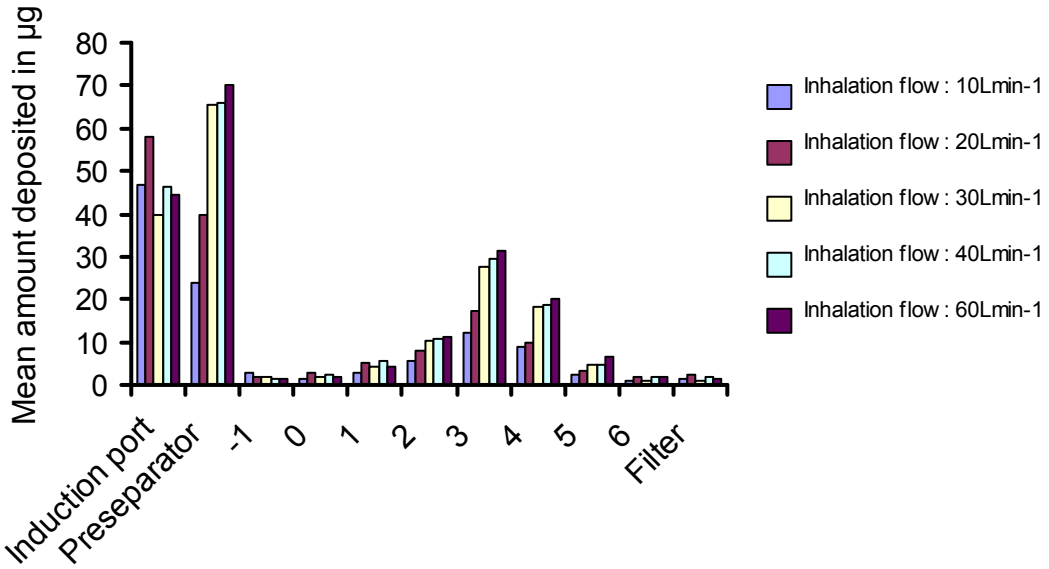


Figure 5.5 Mean amounts (µg) of salbutamol from the Accuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flow (10-60) $Lmin^{-1}$ following two inhalations per dose using a 4L inhaled volume

Table 5.6 Mean (SD) Dose emission of salbutamol from the Easyhaler® using the ACI at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	52.43(8.6)	25.16(2.7)	33.61(3.4)	37.07(7.7)	20.82(1.0)
Preseparator	13.13(2.8)	69.13(7.3)	74.71(3.4)	87.29(11.0)	118.11(4.9)
-1	0.53(0.3)	0.86(1.0)	0.75(0.4)	0.71(0.4)	1.36(0.3)
0	0.44(0.1)	0.92(0.8)	1.72(1.0)	0.95(0.6)	1.84(0.5)
1	0.59(0.1)	1.74(1.4)	2.76(1.2)	2.43(0.7)	3.77(0.6)
2	0.99(0.2)	5.57(2.5)	5.71(0.5)	6.55(0.9)	8.23(0.6)
3	1.86(0.1)	13.60(4.2)	16.98(1.6)	20.21(1.1)	23.79(0.7)
4	1.46(0.2)	10.38(1.5)	15.36(2.8)	17.83(1.1)	18.22(0.6)
5	0.78(0.2)	1.92(0.8)	4.44(0.7)	5.60(0.9)	4.63(1.0)
6	0.26(0.1)	0.68(0.5)	1.20(0.3)	0.96(0.6)	1.90(0.2)
Filter	0.24(0.1)	0.60(0.4)	1.47(0.6)	2.62(1.9)	2.38(1.1)
TED (µg)	72.72(9.5)	130.17(12.4)	158.72(7.2)	182.10(13.1)	205.01(6.4)
TED (% nominal dose)	36.36(4.8)	64.89(6.2)	79.40(3.8)	91.00(6.5)	102.20(3.3)
FPD (µg)	2.63(0.5)	23.49(2.3)	45.90(5.2)	54.58(3.3)	60.77(1.7)
FPD (% Emitted dose)	3.69(0.2)	18.16(2.4)	28.91(2.2)	30.08(2.6)	29.66(1.0)
FPD (% nominal dose)	1.30(0.9)	11.741.1)	22.9(2.6)	27.3(1.6)	30.4(0.8)
MMAD (µm)	6.3(0.2)	4.2(0.3)	2.3(0.1)	2.2(0.1)	2.3(0.1)
GSD	2.2(0.2)	1.6(0.3)	1.8(0.2)	1.6(0.1)	1.7(0)

Table 5.7 Mean (SD) Dose emission of salbutamol from the Easyhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalation per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	48.54(4.1)	45.25(4.0)	47.13(5.2)	45.80(11.0)	35.70(5.5)
Preseparator	34.65(12.8)	59.59(4.2)	72.19(2.9)	72.77(3.8)	88.14(4.6)
-1	1.22(0.2)	1.70(0.4)	1.14(0.2)	6.27(1.8)	2.47(1.1)
0	2.02(0.3)	1.27(0.2)	1.49(0.6)	2.89(0.3)	2.99(0.8)
1	2.96(0.4)	2.07(0.4)	2.65(0.4)	4.76(0.5)	4.51(0.5)
2	5.33(0.4)	5.66(1.2)	5.24(0.4)	9.06(0.8)	8.60(0.6)
3	6.35(0.4)	13.59(0.4)	18.43(0.4)	20.62(1.0)	24.31(1.3)
4	4.21(0.5)	10.61(0.2)	15.67(0.9)	16.92(1.2)	19.17(0.7)
5	1.52(1.3)	2.75(0.2)	4.50(0.2)	5.56(0.8)	4.85(0.1)
6	0.35(0.1)	1.56(0.2)	1.46(0.2)	2.99(0.5)	2.28(0.1)
Filter	0.36(0.1)	1.21(.8)	2.10(0.8)	3.39(0.8)	2.55(1.2)
TED (µg)	107.51(16.4)	145.13(4.7)	172.01(8.0)	191.10(10.5)	195.56(1.3)
TED (% nominal dose)	53.75(8.2)	72.56(2.3)	86.00(4.0)	95.55(5.3)	97.78(0.6)
FPD (µg)	6.20(1.0)	25.87(1.6)	43.22(0.7)	54.33(1.1)	63.28(2.2)
FPD (% Emitted dose)	5.78(0.5)	17.73(1.2)	25.16(0.9)	28.59(1.1)	32.36(1.1)
FPD (% nominal dose)	3.10(0.5)	12.93(0.8)	21.61(0.4)	27.16(0.6)	31.6(0.2)
MMAD (µm)	7.7(0.4)	4.2(0.1)	3.2(0.1)	3.0(0.1)	2.4(0.0)
GSD	1.7(0.1)	1.8(0.0)	1.7(0.1)	2.1(0.1)	1.9(0.2)

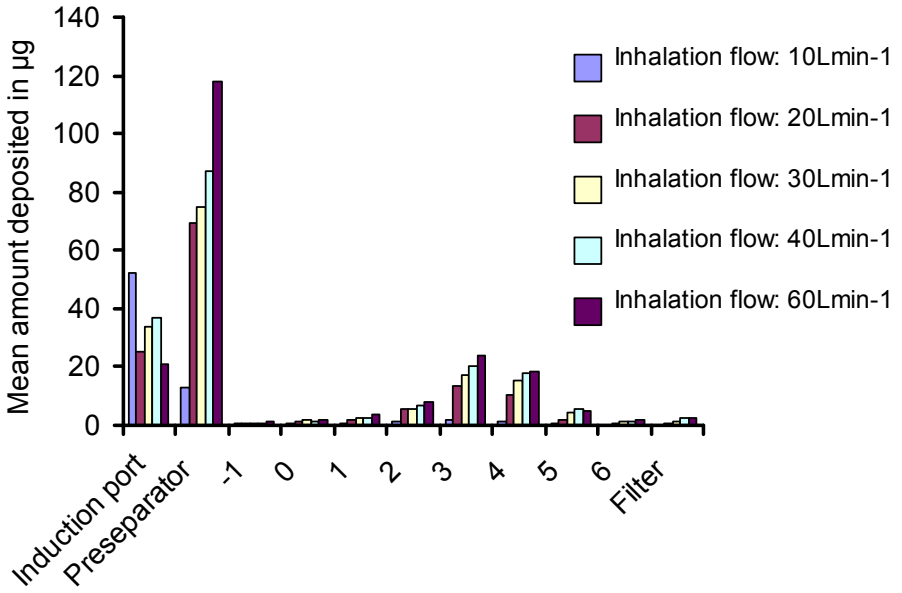


Figure 5.6 Mean amounts (µg) of salbutamol from the Easyhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 2L inhaled volume

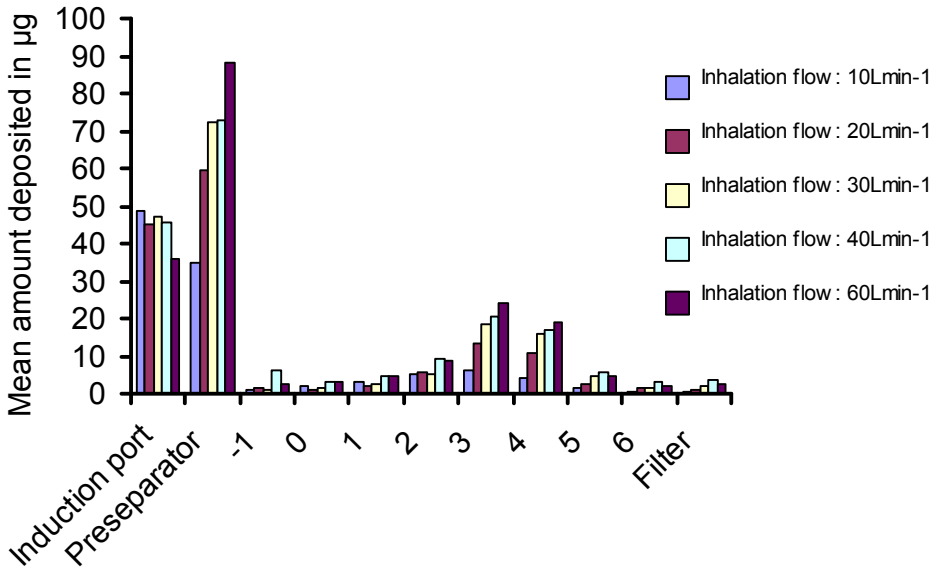


Figure 5.7 Mean amounts (µg) of salbutamol from the Easyhaler deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 2L inhaled volume

Table 5.8 Mean (SD) Dose emission of salbutamol from the Easyhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	63.88(7.7)	34.25(1.4)	26.23(2.8)	28.91(1.1)	19.82(1.9)
Preseparator	2.10(0.8)	63.25(10.6)	88.96(5.9)	80.82(3.4)	107.67(2.3)
-1	1.32(0.3)	2.37(0.2)	0.97(0.3)	0.87(0.4)	1.09(0.2)
0	0.67(0.3)	2.15(0.6)	1.08(0.3)	1.60(0.6)	1.98(0.6)
1	0.63(0.2)	2.69(0.5)	3.02(0.3)	3.32(0.7)	3.44(0.2)
2	1.42(0.2)	6.10(1.1(	6.78(1.2)	7.11(1.0)	7.89(0.7)
3	2.35(0.4)	16.06(1.5)	18.09(1.3)	21.51(2.4)	26.95(0.9)
4	1.75(0.6)	10.99(1.6)	15.03(1.1	19.48(3.0)	20.74(0.9)
5	0.96(0.4)	4.73(0.7)	4.15(0.5)	5.56(1.5)	5.63(0.5)
6	0.46(0.2)	2.24(0.7)	1.28(1.6)	2.16(0.4)	1.91(0.2)
Filter	0.43(0.2)	1.96(0.1)	3.04(0.4)	2.70(0.9)	2.29(0.7)
TED (µg)	75.97(8.2)	146.78(14.1)	168.66(7.3)	174.04(8.4)	199.40(1.1)
TED (% nominal dose)	37.99(4.1)	73.39(7.0)	84.26(3.6)	87.00(4.2)	101.20(2.5)
FPD (µg)	3.46(0.9)	31.45(4.4)	49.52(2.3)	59.79(6.0)	66.73(2.4)
FPD (% Emitted dose)	4.57(1.2)	21.43(0.8)	29.38(1.3)	34.30(2.0)	33.57(1.3)
FPD (% nominal dose)	1.70(.0.5)	15.73(2.2)	24.8(1.2(	29.9(3.0)	33.4(1.2)
MMAD (µm)	6.8(0.3)	4.2(0.1)	2.3(0.1(	2.2(0.1)	2.3(0.0)
GSD	2.6(0.3)	1.9(0.1)	1.7(0.1)	1.7(0.1)	1.6(0.0)

Table 5.9 Mean (SD) Dose emission of salbutamol from the Easyhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalation per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	64.44(4.5)	26.55(2.4)	41.34(2.9)	36.76(6.9)	33.51(3.7)
Preseparator	14.72(2.8)	69.21(2.0)	71.05(6.8)	85.00(9.9)	87.71(3.0)
-1	0.70(0.4)	1.32(0.9)	6.64(2.2)	5.79(2.8)	2.04(0.2)
0	0.93(0.7)	0.83(0.5)	3.26(0.5)	4.38(1.6)	5.56(0.5)
1	1.53(0.1)	1.98(0.9)	4.22(0.2)	3.97(0.3)	1.30(0.3)
2	2.64(0.2)	4.65(2.0)	6.90(0.4)	9.55(0.4)	9.48(0.5)
3	5.18(0.4)	15.64(0.5)	18.83(0.7)	23.18(1.1	25.62(0.5)
4	2.61(1.2)	12.77(1.2)	15.02(2.0)	19.16(1.2)	19.62(0.5)
5	1.12(0.5)	3.65(0.4)	4.68(0.6)	4.34(1.2)	5.83(1.1)
6	0.70(0.5)	1.01(0.4)	3.01(0.4)	2.20(0.7)	2.28(0.4)
Filter	0.63(0.4)	1.13(0.3)	3.02(0.6)	3.02(0.7)	2.57(0.9)
TED (µg)	95.19(8.3)	138.95(2.8)	178.01(4.8)	197.33(6.1)	195.52(3.0)
TED (% nominal dose)	47.60(4.2)	69.60(1.4)	80.22(1.0)	98.67(3.1)	97.76(1.5)
FPD (µg)	4.78(1.3)	40.14(1.2)	41.98(9.2)	57.95(0.7)	65.82(1.9)
FPD (% Emitted dose)	4.97(1.0)	28.89(0.6)	21.52(10.0)	29.36(0.8)	33.67(1.0)
FPD (% nominal dose)	2.40(0.7)	20.07(0.8)	20.99(4.6)	28.98(0.4)	32.9(0.9)
MMAD (µm)	6.7(0.2)	4.2(0.2)	3.3(0.4)	3.1(0.2)	2.4(0.0)
GSD	1.7(0.0)	1.9(0.1)	1.9(0.3)	2.2(0.3)	1.7(0.1)

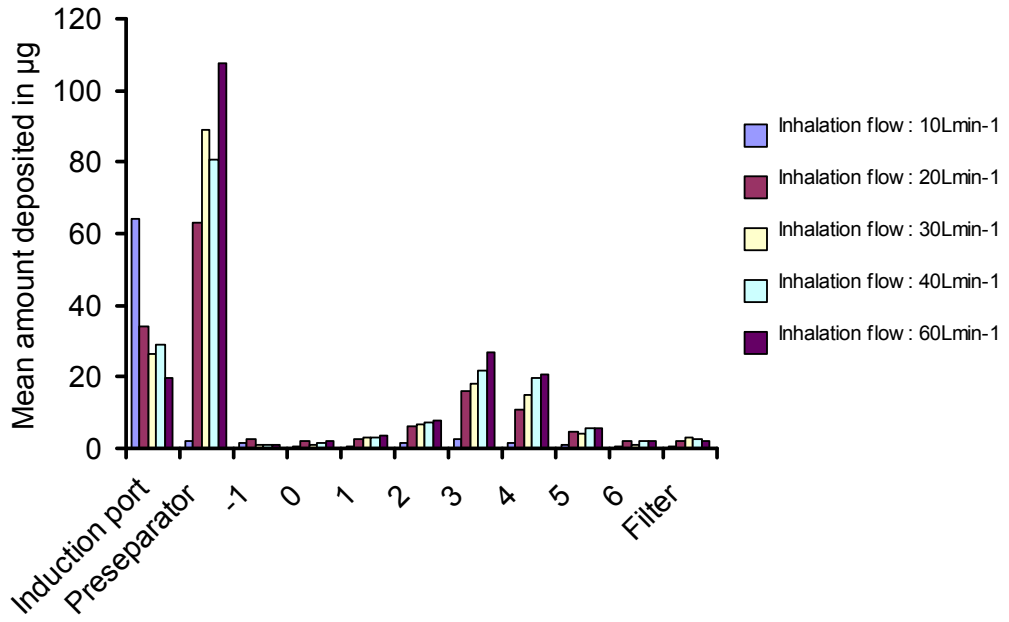


Figure 5.8 Mean amounts (µg) of salbutamol from the Easyhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume

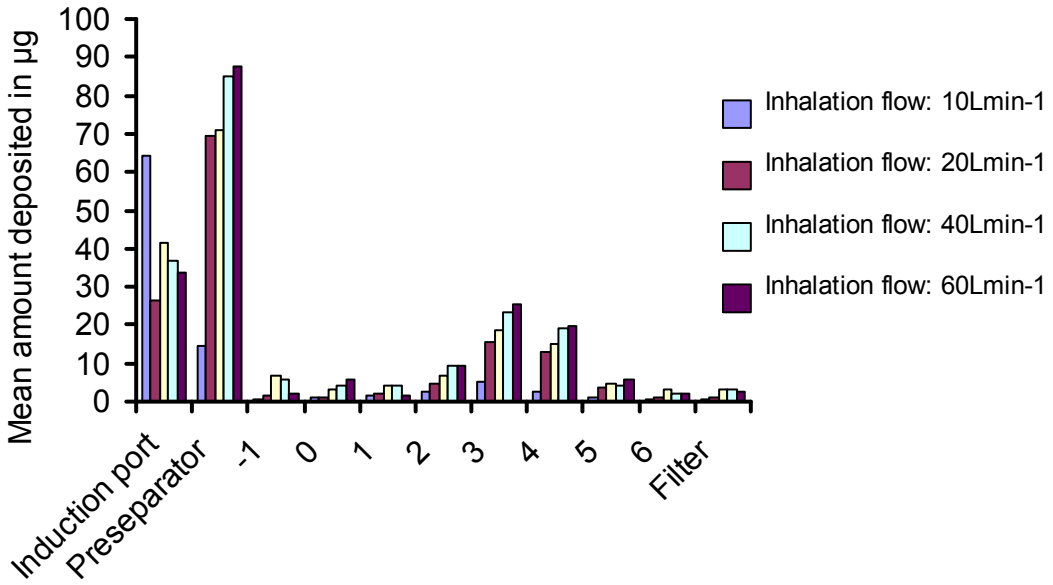


Figure 5.9 Mean amounts (µg) of salbutamol from the Easyhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 4L inhaled volume

Table 5.10 Mean (SD) Dose emission of salbutamol from the Clickhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	27.89(3.3)	20.15(2.9)	14.45(0.7)	13.80(1.3)	12.44(1.0)
Preseparator	1.56(0.1)	18.98(3.3)	33.14(0.6)	34.57(1.1)	40.51(2.5)
-1	0.22(0.1)	0.27(0.1)	0.67(0.4)	0.68(0.2)	1.10(0.1)
0	0.32(0.2)	0.79(0.2)	1.05(0.2)	3.39(1.6)	1.96(0.2)
1	0.56(0.2)	2.54(0.5)	3.96(0.4)	3.19(1.4)	5.72(0.3)
2	0.94(0.2)	4.36(0.6)	7.91(0.6)	7.54(0.3)	9.41(0.9)
3	1.91(0.9)	6.42(0.7)	12.38(0.8)	12.99(0.3)	17.72(0.9)
4	1.71(0.7)	3.51(0.4)	6.57(0.4)	7.30(1.0)	9.45(0.4)
5	0.85(0.2)	1.12(0.3)	2.25(0.7)	2.67(0.4)	2.86(0.8)
6	0.45(0.2)	0.61(0.2)	1.15(0.1)	1.20(0.1)	1.93(0.5)
Filter	0.78(0.1)	0.95(0.6)	2.02(0.7)	2.26(0.9)	1.00(0.6)
TED (µg)	37.18(2.9)	59.69(3.6)	85.55(2.4)	89.59(2.3)	103.94(1.0)
TED (% nominal dose)	32.61(2.6)	52.20(3.3)	75.05(2.1)	78.40(2.2)	91.18(0.9)
FPD (µg)	3.70(0.9)	10.76(1.5)	26.71(1.3)	31.21(1.4)	44.40(1.5)
FPD (% Emitted dose)	9.95(2.6)	18.03(2.6)	31.08(1.2)	34.84(0.8)	42.82(1.4)
FPD (% nominal dose)	3.2(0.8)	9.44(1.3)	23.4(1.2(	27.4(1.3)	39.0(1.3)
MMAD (µm)	5.2(0.5)	4.8(0.1)	3.80(0.2)	3.30(0.0)	2.7(0.1)
GSD	2.1(0.4)	1.7(0.0)	1.7(0.0)	1.8(0.1)	1.8(0.1)

Table 5.11 Mean (SD) Dose emission of salbutamol from the Clickhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD amount deposited on each stage (µg)				
	Inhalation flow (Lmin-1)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	15.30(2.3)	24.47(1.3)	35.21(1.6)	15.29(2.4)	12.51(1.7)
Preseparator	7.72(2.3)	17.30(1.6)	13.12(0.8)	32.75(1.6)	36.71(6.3)
-1	0.85(0.3)	1.63(0.7)	0.76(0.0)	1.21(0.7)	0.48(0.5)
0	0.93(0.1)	1.48(0.1)	2.22(0.1)	2.11(1.1)	1.07(0.6)
1	1.23(0.4)	2.66(0.2)	3.19(0.1)	4.34(0.2)	4.68(0.7)
2	2.06(0.2)	4.38(0.4)	6.51(0.2)	8.04(0.5)	10.30(0.9)
3	4.95(0.5)	6.98(0.7)	12.65(0.6)	13.90(0.7)	18.71(1.3)
4	2.55(0.3)	3.95(0.3)	6.63(0.6)	7.26(0.7)	11.07(0.9)
5	1.50(0.3)	1.22(0.2)	1.87(0.2)	2.98(0.8)	3.05(0.5)
6	0.84(0.1)	0.92(0.1)	1.21(0.3)	1.83(0.4)	1.12(1.2)
Filter	0.65(0.1)	0.72(0.1)	0.99(0.4)	2.19(0.6)	0.88(0.6)
TED (µg)	38.59(4.0)	65.72(1.2)	84.19(1.9)	91.79(1.5)	100.59(7.4)
TED (% nominal dose)	33.85(3.5)	57.65(1.0)	73.85(1.7)	80.52(1.3)	88.20(6.4)
FPD (µg)	5.49(0.5)	15.26(0.8)	24.89(0.4)	33.26(1.9)	46.98(1.8)
FPD (% Emitted dose)	14.46(1.9)	23.22(1.0)	29.52(0.9)	36.21(1.8)	46.71(3.0)
FPD (% nominal dose)	4.8(0.5)	13.4(0.7)	21.8(0.4)	29.2(1.7)	41.2(1.5)
MMAD (µm)	6.5(0.3)	4.8(0.1)	3.9(0.1)	3.3(0.1)	2.6(0.0)
GSD	2.02(0.1)	1.9(0.1)	1.8(0.0)	1.8(0.0)	1.6(0.0)

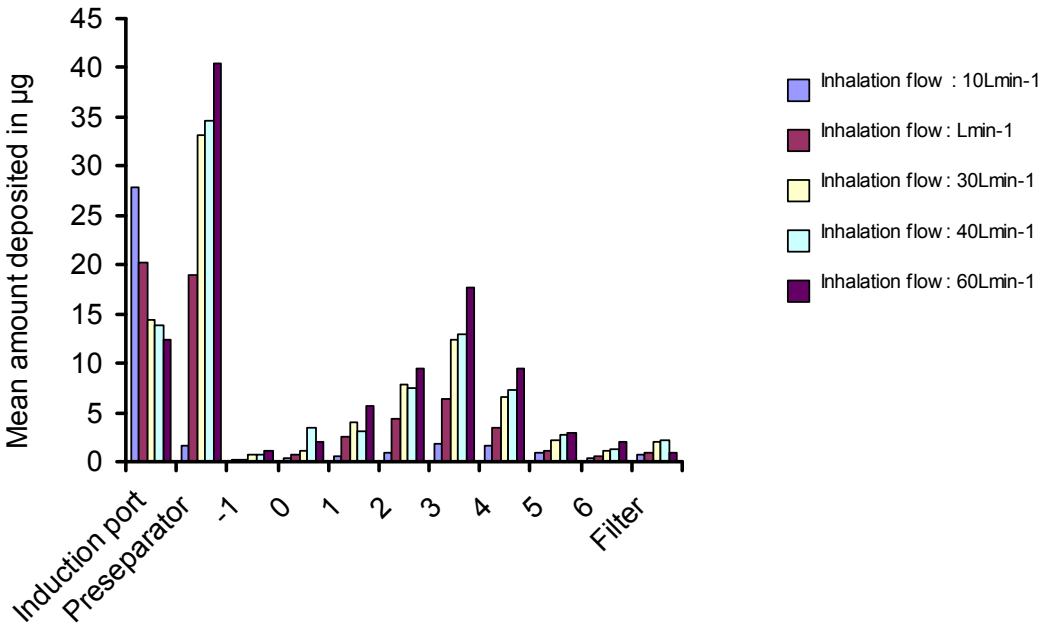


Figure 5.10 Mean amounts (µg) of salbutamol from the Clickhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flow (10-60) $Lmin^{-1}$ following one inhalation per dose using a 2L inhaled volume

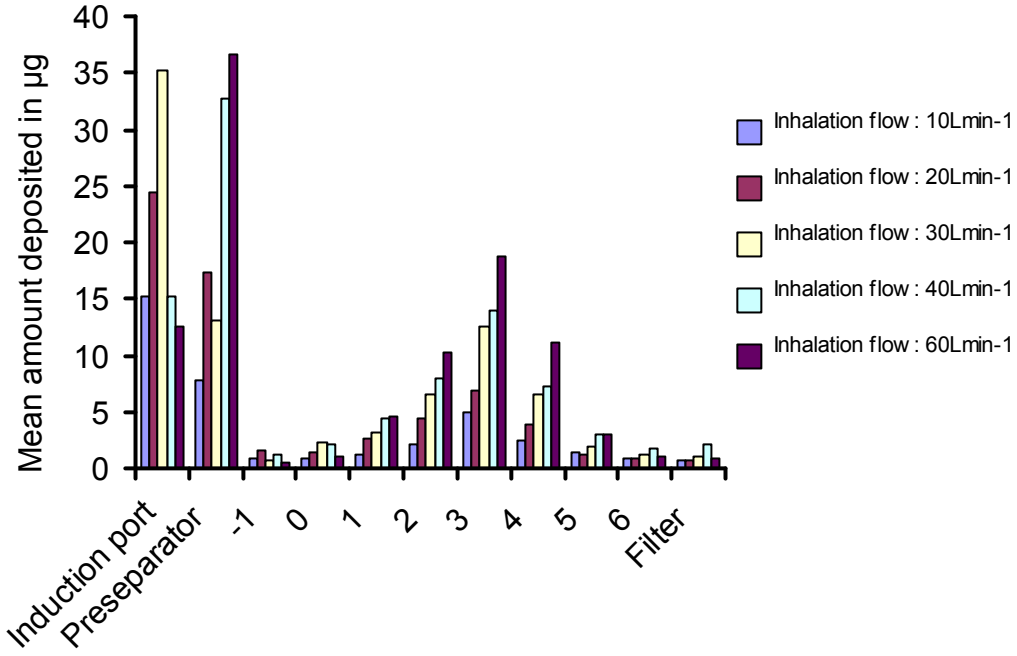


Figure 5.11 Mean amounts (µg) of salbutamol from the Clickhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 2L inhaled volume

Table 5.12 Mean (SD) Dose emission of salbutamol from the Clickhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD amount deposited on each stage (µg)				
	Inhalation flow ($Lmin^{-1}$)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	9.08(2.0)	14.77(2.3)	13.98(0.7)	15.30(1.8)	14.51(2.3)
Preseparator	2.26(0.7)	31.22(1.5)	38.78(1.3)	35.55(2.1)	38.71(1.9)
-1	2.36(1.5)	0.48(0.1)	0.57(0.1)	0.84(0.2)	1.60(0.1)
0	5.73(2.5)	1.01(0.1)	1.13(0.2)	1.84(0.4)	1.45(0.4)
1	1.86(0.7)	3.99(0.6)	4.71(0.3)	5.30(0.5)	5.01(0.6)
2	2.11(1.2)	7.54(0.4)	8.48(0.7)	9.16(0.5)	9.07(0.4)
3	4.49(1.5)	12.16(0.9)	14.11(0.3)	17.03(0.9)	18.37(0.3)
4	2.82(0.5)	6.51(0.6)	7.31(1.2)	8.41(0.4)	9.76(0.4)
5	2.81(1.4)	1.52(0.4)	2.38(0.3)	2.54(0.3)	2.80(0.6)
6	2.39(1.4)	1.01(0.2)	1.30(0.2)	1.05(0.5)	1.62(0.1)
Filter	1.92(0.9)	2.55(0.6)	2.18(0.6)	1.53(0.7)	1.52(0.6)
TED (µg)	37.74(6.5)	82.78(3.2)	94.93(3.8)	98.54(3.8)	104.20(4.6)
TED (% nominal dose)	33.10(5.7)	72.20(3.1)	83.40(3.1)	87.80(5.4)	91.40(4.1)
FPD (µg)	9.06(1.9)	20.28(1.0)	29.35(1.4)	36.44(2.1)	44.94(0.8)
FPD (% Emitted dose)	24.15(3.9)	24.50(1.5)	30.92(0.8)	36.97(0.9)	42.88(2.0)
FPD (% nominal dose)	8.0(1.7)	17.8(0.9)	25.7(1.2)	31.96(1.8)	39.4(0.7)
MMAD (µm	7.6(1.3)	4.7(0.1)	3.8(0.0)	3.4(0.1)	2.7(0.0)
GSD	2.5(0.3)	1.7(0.1)	1.7(0.0)	1.7(0.0)	1.8(0.1)

Table 5.13 Mean (SD) Dose emission of salbutamol from the Clickhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD amount deposited on each stage (µg)				
	Inhalation flow ($Lmin^{-1}$)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	14.31(1.4)	30.10(1.0)	38.01(1.2)	34.62(1.3)	13.35(2.9)
Preseparator	6.99(2.1)	17.50(1.1(	14.58(0.3)	17.84(1.9)	37.17(5.0)
-1	1.36(0.3)	1.14(0.5)	0.77(0.2)	1.75(0.8)	0.50(1.1)
0	0.93(0.1)	2.03(0.7)	1.99(0.3)	1.38(0.1)	1.22(0.1)
1	1.66(0.4)	4.46(0.3)	3.59(0.2)	4.58(0.9)	4.37(0.4)
2	3.44(0.8)	7.09(0.3)	7.30(0.7)	9.82(0.7)	9.61(0.8)
3	8.07(0.2)	13.53(0.4)	14.66(0.2)	17.15(0.3)	19.43(1.4)
4	2.74(0.5)	6.83(0.4)	8.28(0.3)	9.21(0.5)	11.13(0.5)
5	1.52(0.4)	2.26(0.4)	2.45(0.1)	2.99(0.6)	3.12(0.4)
6	0.70(0.4)	1.77(0.5)	1.64(0.3)	1.53(0.4)	1.23(1.2)
Filter	0.45(0.2)	2.00(0.2)	1.35(0.1)	1.39(0.4)	1.09(0.7)
TED (µg)	42.05(5.2)	88.71(2.9)	94.62(1.5)	102.26(1.7)	102.22(3.7)
TED (% nominal dose)	36.89(4.6)	77.82(2.6)	83.00(1.4)	89.70(1.5)	89.80(3.3)
FPD (µg)	5.29(1.0)	22.52(1.1)	30.25(0.4)	38.66(1.0)	47.33(2.4)
FPD (% Emitted dose)	12.48(1.2)	25.44(0.9)	31.84(0.6)	38.21(1.3)	46.37(0.9)
FPD (% nominal dose)	4.6(0.9)	19.8(1.0)	26.5(0.3)	33.9(0.9)	41.5(2.1)
MMAD (µm)	6.8(0.1)	4.7(0.1)	3.7(0.1)	3.4(0.1)	2.6(0.0)
GSD	1.8(0.1)	1.8(0.1)	1.8(0.1)	1.7(0.0)	1.6(0.1)

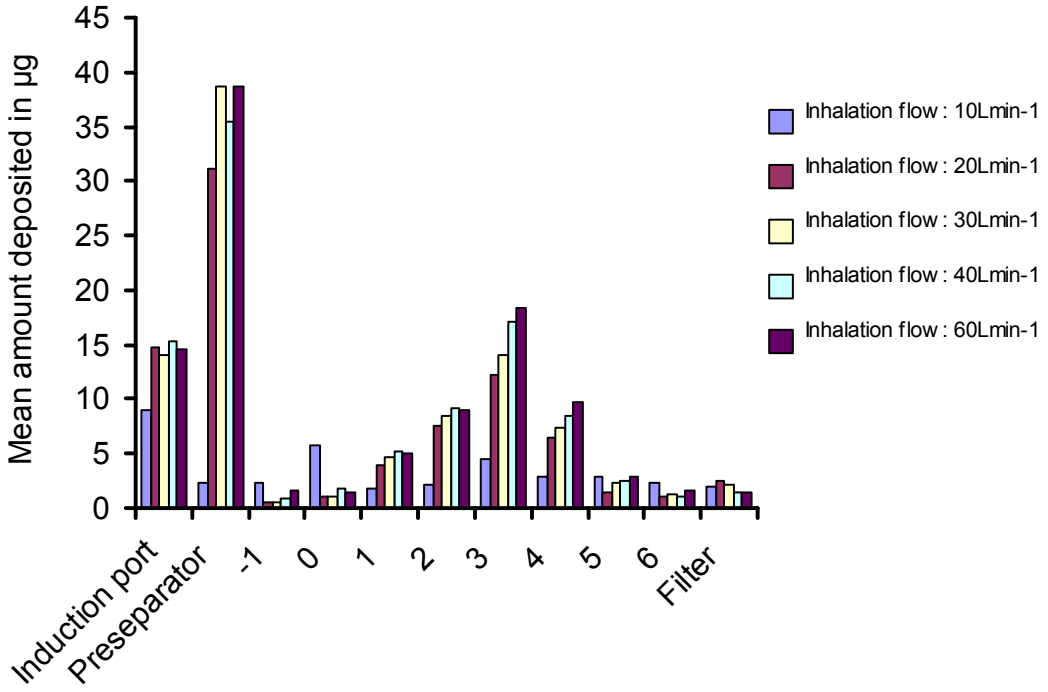


Figure 5.12 Mean amounts (µg) of salbutamol from the Clickhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume

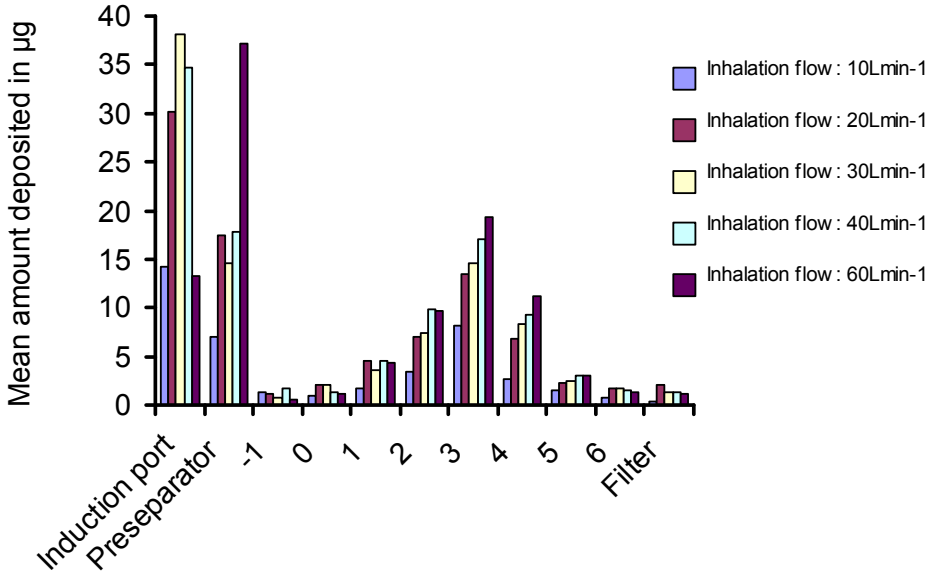


Figure 5.13 Mean amounts (µg) of salbutamol from the Clickhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 4L inhaled volume (n=5)

Table 5.14 Mean (SD) Dose emission of terbutaline from the Turbuhaler® using the ACI at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow ($Lmin^{-1}$)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	40.78(13.6)	145.13(20.3)	147.3(14.3)	145.5(5.3)	124.38(23.4)
Preseparator	18.95(9.8)	76.4(12)	88.1(14.2)	74.08(8.2)	81.29(12.4)
-1	2.19(1.7)	7.24(1.6)	7.44(3.4)	7.82(2.2)	7.74(3.0)
0	7.30(2.6)	21.47(1.8)	22.2(2.1)	18.80(6.2)	16.34(7.0)
1	10.52(2.6)	24.06(1.0)	26.3(3)	29.46(2.2)	31.61(6.4)
2	13.50(3.4)	22.85(1.0)	27.67(3)	32.56(1.4)	38.30(8.4)
3	16.34(3.2)	23.25(0.7)	35.19(3.1)	36.89(1.3)	46.47(7.6)
4	9.61(1.5)	12.08(0.9)	13.65(1.3)	24.87(1.9)	26.91(9.5)
5	4.95(1.0)	5.41(0.16)	5.6(1.1)	6.98(2.6)	9.55(4.3)
6	2.41(0.4)	5.0(0.5)	4.31(0.4)	5.41(2.1)	4.77(1.9)
Filter	0.88(0.5)	4.5(1.9)	6.14(1.5)	6.15(1.4)	2.87(1.7)
TED (µg)	127.44(23.4)	347.22(28.2)	383.92(5.0)	388.54(2.9)	383.92(8.9)
TED (%nominal dose)	25.49(4.7)	69.45(5.6)	76.2(0.8)	77.8(0.4)	76.23(0.8)
FPD (µg)	17.30(2.0)	81.0(4.1)	101.34(5)	122.51(6.7)	167.78(19.9)
FPD (% Emitted dose)	14.03(2.6)	23.4(1.7)	26.4(1.3	31.5(1.9)	44.18(9.6)
FPD (%nominal dose)	3.46(0.4)	16.2(0.8)	20.27(1.0)	24.50(1.3)	33.56(4.0)
MMAD	8.0(0.7)	4.0(0.1)	3.8(0.2)	3.5(0)	2.2(0.1)
GSD	1.7(0.1)	1.7(0.1)	1.7(1.0)	1.7(0.1)	1.7(0.1)

Table 5.15 Mean (SD) Dose emission of terbutaline from the Turbuhaler® using the ACI at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 2L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow ($Lmin^{-1}$)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	67.58(40)	108.89(3.4)	105.05(4.5)	109.42(8.7)	125.76(5.1)
Preseparator	36.62(3.5)	46.41(6.2)	52.62(7.7)	44.387.7)	49.31(3.9)
-1	6.20(3.1)	7.96(1.0)	11.34(3.4)	7.26(2.1)	6.48(1.3)
0	14.02(2.4)	17.01(2.9)	12.93(2.8)	12.62(4.2)	13.18(2.3)
1	20.50(3.5)	31.85(1.9)	31.69(0.9)	25.75(11.3)	39.23(4.4)
2	23.87(5.5)	37.25(2.0)	40.52(2.3)	40.38(4.5)	49.03(5.6)
3	28.55(5.3)	49.93(6.5)	57.35(2.5)	60.83(3.4)	69.62(1.7)
4	17.97(5.7)	33.89(4.0)	38.87(1.7)	40.92(3.8)	48.50(3.3)
5	8.66(3.4)	26.63(2.2)	20.26(1.3)	28.20(3.8)	26.00(9.4)
6	4.08(1.5)	6.34(1.7)	6.57(1.0)	10.15(5.3)	8.19(2.1)
Filter	2.09(0.8)	4.46(2.2)	6.23(12.4)	7.71(4.7)	7.83(5.7)
TED (µg)	230.14(26.6)	370.62(1.7)	383.41(2.5)	392.29(7.5)	443.13(15.7)
TED (% nominal dose)	46.03(5.3)	75.03(1.7)	76.68(4.5)	78.46(1.5)	88.63(3.1)
FPD (µg)	32.00(9.6)	106.97(10.2)	138.60(1.5)	173.94(6.5)	223.47(10.6)
FPD (% Emitted dose)	13.91(2.8)	28.59(8.4)	36.17(0.1)	44.89(2.6)	51.16(2.3)
FPD (% nominal dose)	6.40(1.9)	21.39(1.7)	27.72(0.9)	34.79(1.3)	44.69(2.1)
MMAD (µm)	8.3(0.7)	5.1(0.2)	4.1(0.1)	3.2(0.2)	2.8(0.2)
GSD	1.7(0.1)	1.8(02)	1.8(0.1)	1.8(0.1)	1.8(0.1)

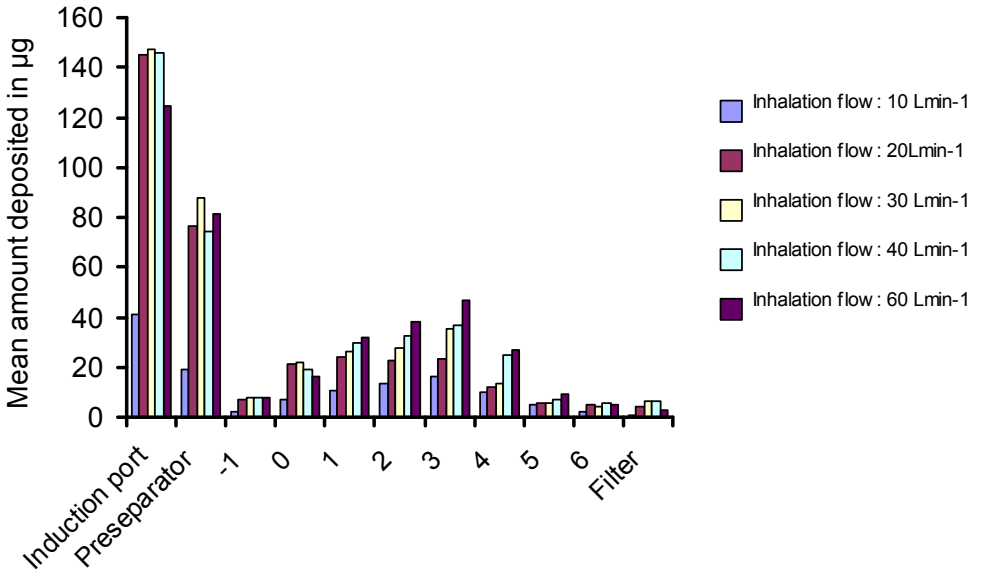


Figure 5.14 Mean amounts (µg) of terbutaline from the Turbuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) Lmin-1 following one inhalation per dose using a 2L inhaled volume

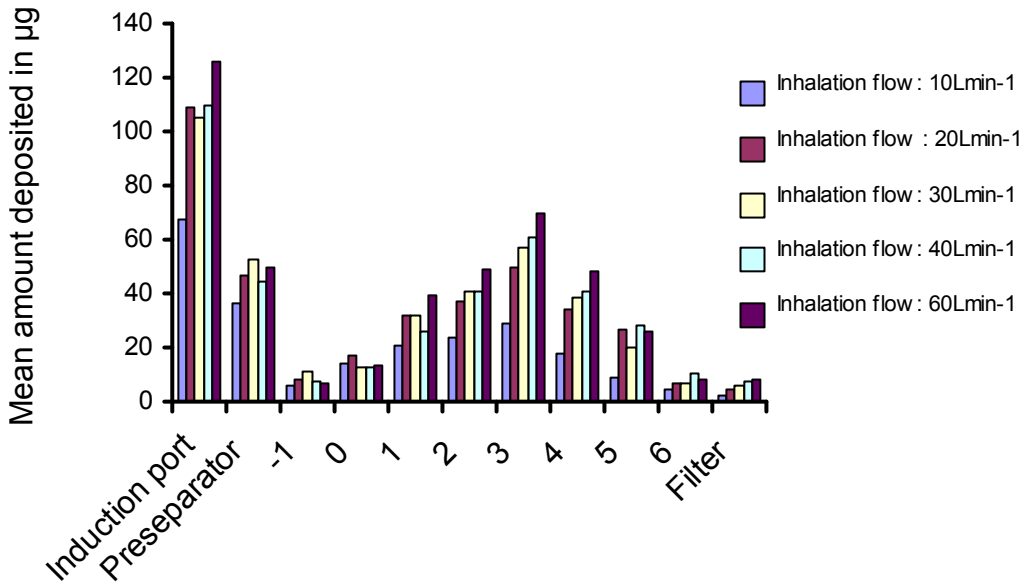


Figure 5.15 Mean **a**mounts (µg) of terbutaline from the Turbuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 2L inhaled volume

Table 5.16 Mean (SD) Dose emission of terbutaline from the Turbuhaler® using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD amount deposited on each stage (µg)				
	Inhalation flow ($Lmin^{-1}$)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	88.24(3.6)	133.24(17.9)	176.87(7.9)	147.98(15)	142.62(14.3)
Preseparator	34.13(3.6)	74.83(20.3)	71.65(3.2)	96.01(21.9)	86.38(5.2)
-1	4.89(1.0)	7.07(1.7)	7.08(4.4)	6.29(2.4)	7.09(1.9)
0	9.63(1.0)	25.15(2.0)	21.98(2.0)	8.77(1.9)	19.15(3.6)
1	10.40(0.7)	24.01(2.1)	24.87(0.7)	24.88(2.7)	35.58(3.8)
2	12.60(1.0)	23.83(1.9)	24.26(1.6)	28.98(2.9)	43.21(2.6)
3	16.54(1.0)	23.48(1.8)	26.33(2.1)	33.31(4.2)	57.94(4.4)
4	12.671.1)	12.01(1.0)	13.20(1.8)	17.00(4.9)	27.56(5.4)
5	7.97(0.6)	6.22(0.9)	4.45(1.4)	5.30(2.0)	14.23(4.8)
6	5.26(0.5)	5.52(1.6)	4.77(2.1)	4.99(2.8)	3.92(1.0)
Filter	1.68(0.2)	5.46(2.4(	4.43(1.2)	7.58(0.9)	2.26(1.7)
TED (µg)	203.99(5.2)	340.80(38.7)	380.18(11.1)	381.08(28)	437.79(26.3)
TED (% nominal dose)	40.80(1.0)	68.22(7.6)	76.00(2.3)	76.27(5.7)	87.56(5.3)
FPD (µg)	26.561.3)	84.43(5.9)	117.15(4.1)	127.40(14.4)	194.84(10.6)
FPD (% Emitted dose)	13.00(0.6)	24.99(5.9)	30.65(0.6)	33.59(4.6)	44.52(1.1)
FPD (% nominal dose)	5.31(0.3)	16.89(1.2)	23.43(0.8)	25.48(2.9)	38.97(2.1)
MMAD (µm)	7.4(0.2)	6.0(0.1)	2.7(0.1)	2.3(0.1)	2.3(0.1)
GSD	2.1(0)	1.6(0.1)	1.68(0.1)	1.62(0.1)	1.7(0.1)

Table 5.17 Mean (SD) Dose emission of terbutaline from the Turbuhaler using the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations per dose using a 4L inhaled volume (n=5). Amount expressed in (µg) unless stated

	Mean (SD) amount deposited on each stage (µg)				
	Inhalation flow ($Lmin^{-1}$)				
Stage	**10**	**20**	**30**	**40**	**60**
Induction port	86.22(16.6)	98.81(7.2)	89.46(9.4)	95.76(3.9)	131.12(3.9)
Preseparator	38.87(4.2)	30.39(4.7)	37.54(5.8)	32.13(2.1)	48.47(3.4)
-1	8.15(1.8)	8.41(0.9)	8.91(1.4)	10.06(1.4)	7.85(2.4)
0	13.07(1.2)	15.44(2.3)	15.88(3.0)	15.24(1.2)	18.99(3.1))
1	23.90(3.4)	33.06(3.7)	33.13(3.6)	34.62(1.1)	36.79(2.8)
2	26.00(5.3)	42.28(1.5)	38.47(3.2)	45.15(1.1)	53.35((3.5)
3	33.82(10.4)	63.74(2.4)	65.41(2.9)	66.45(1.7)	79.96(2.3)
4	22.17(5.6)	39.90(3.1)	39.30(5.2)	42.76(1.1)	57.03(1.2)
5	11.24(3.0)	23.08(3.1)	28.50(0.5)	27.89(2.3)	27.15(11.7)
6	3.09(2.1)	9.23(2.3)	19.04(1.9)	19.30(3.1)	7.07(2.3)
Filter	1.85(0.9)	9.76(2.3)	7.85(1.1)	10.18(9.81	5.64(1.2)
TED (µg)	268.40(39.4)	374.10(6.9)	383.50(11.3)	399.53(1.8)	473.41(11.7)
TED (% nominal dose)	53.68(7.9)	74.82(1.4)	76.70(2.3)	79.91(4.5)	94.68(2.3)
FPD (µg)	39.95(10.2)	130.40(8.3)	169.18(8.3)	194.40(0.7)	239.14(15.3)
FPD (% Emitted dose)	14.34(2.3)	34.59(2.0)	44.13(2.1)	48.66(0.1)	51.64(2.8)
FPD (% nominal dose)	7.992.0)	26.08(1.7)	33.84(1.7)	38.88(0.9)	47.83(3.1)
MMAD (µm)	7.8(0.8)	4.8(0.2)	3.7(0.2)	3.2(0.1)	2.8(0)
GSD	1.8(0.1)	1.9(0)	1.94(0.1)	1.90(0)	1.9(0.1)

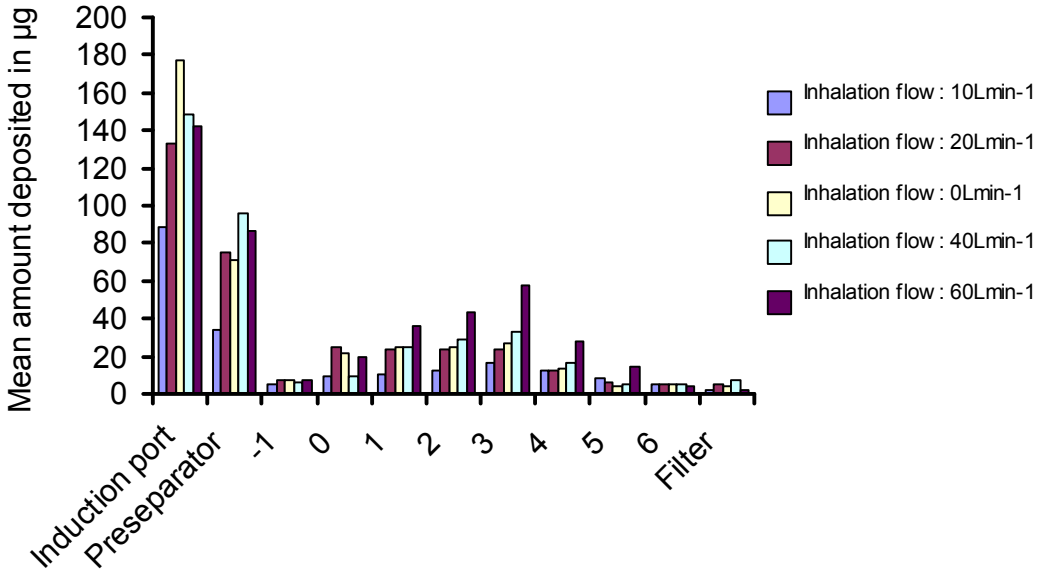


Figure 5.16 Mean amounts (µg) of terbutaline from the Turbuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per dose using a 4L inhaled volume

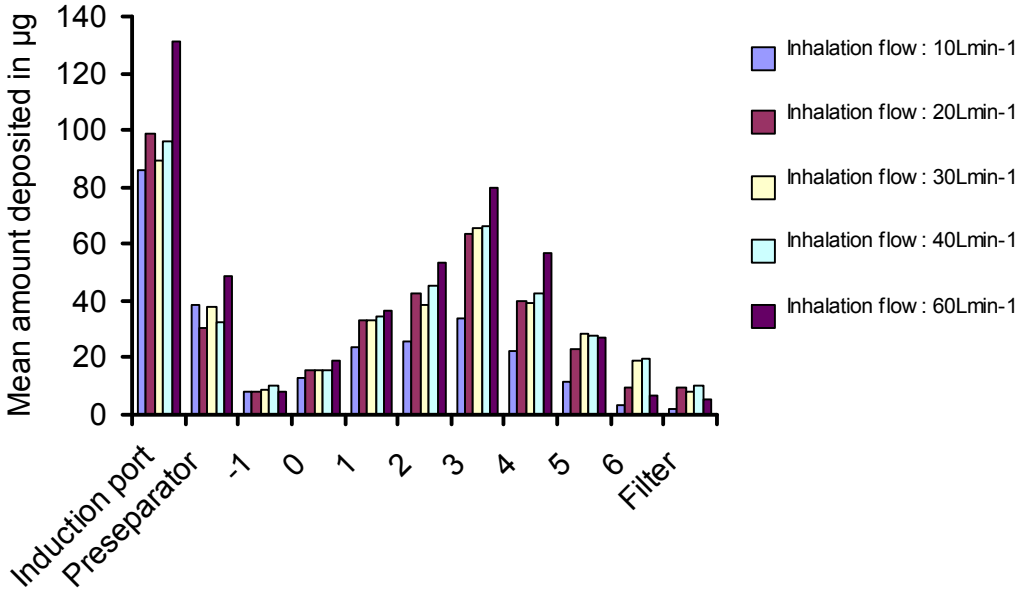


Figure 5.17 Mean amounts (µg) of terbutaline from the Turbuhaler® deposited on each stage of the Andersen Cascade Impactor at different inhalation flows (10-60) $Lmin^{-1}$ following two inhalations using a 4L inhaled volume

A summary of the mean (SD) fine particle dose, expressed as % nominal dose, from the four dry powder inhalers at varying inhalation flows (10-60 $Lmin^{-1}$) using 2L and 4L inhalation volumes following one and two inhalations per metered dose respectively is shown in Tables 5.18. Figures 5.18 to 5.21 describe the variation of the fine particle dose (FPD) from the Accuhaler®, the Easyhaler®, the Clickhaler® and Turbuhaler® with respect to the inhalation flow under the same inhalation volumes and the number of inhalations. The FPD increased with an increase of the inhalation flow, though this varied from device to device.

Tables 5.19 and 5.20 show the statistical comparison of the fine particle dose (% nominal dose) of the four different dry powder inhalers at varying inhalation flows (10-60) $Lmin^{-1}$ using a 2L and a 4L inhaled volumes.

A summary of the mean (SD) MMAD of the drug particles emitted from the four dry powder inhalers at varying inhalation flows (10-60 $Lmin^{-1}$) using 2L and 4L inhalation volumes following one and two inhalations per metered dose respectively is shown in Tables 5.21. From the data presented in Table 5.21 the minimum inhalation flow at which the emitted fine particles with the MMAD of less than 5µm that have the greatest potential for lung deposition from the Accuhaler®, Easyhaler®, and Clickhaler® was about $20Lmin^{-1}$, while that from the Turbuhaler was about $30Lmin^{-1}$.

Figures 5.22 to 5.25 describe the variation of the MMAD of the drug particles emitted from the inhalers with respect to the inhalation flow under the same inhalation volumes and the number of inhalations. Generally, the MMAD decreased with an increase of the inhalation flow.

Table 5.18 Mean (SD) fine particle dose, as % nominal dose from the four different dry powder inhalers following one and two inhalations per metered dose for 2L and 4L inhalation volumes

	Mean (SD) fine particle dose (% nominal dose) following one inhalation							
Device	Accuhaler		Easyhaler		Clickhaler		Turbuhaler	
Inhalation volume	2L	4L	2L	4L	2L	4L	2L	4L
Inhalation flow (Lmin-1)								
10	4.08(1.6)	6.54(0.8)	1.32(0.2)	1.73(0.4)	3.07(0.8)	7.95(1.7)	3.46(0.4)	5.31(0.3)
20	11.59(1)	14.91(1.6)	11.74(1.1)	20.07(2.2)	9.44(1.3)	17.79(0.9)	16.2(0.8)	16.89(1.2)
30	23.89(1.4)	26.76(0.)	22.9(2.6)	24.76(1.2)	21.83(0.4)	25.75(1.2)	20.27(1.0)	23.43.(0.8)
40	29.87(0.8)	31.8(2.4)	27.29(1.6)	29.89(3)	27.38(1.3)	31.96(1.8)	24.50(1.3)	25.48(2.2.9)
60	35.95(1.1)	37.19(1.9)	30.39(0.8)	33.37(1.2)	38.95(1.55)	39.42(2.1)	33.56(4.0)	38.97(2.1)
	Mean (SD) fine particle dose (% nominal dose) following two inhalations							
Device	Accuhaler		Easyhaler		Clickhaler		Turbuhaler	
Inhalation volume	2L	4L	2L	4L	2L	4L	2L	4L
Inhalation flow (Lmin-1)								
10	3.63(0.7)	6.30(1.0)	3.10(0.5)	2.39(0.7)	4.82(0.5)	4.64(0.9)	6.40(1.9)	7.99(2.0)
20	14.45(0.3)	17.28(2.6)	12.93(0.8)	15.73(0.6)	13.39(0.7)	19.75(1)	21.39(1.7)	26.08(1.7)
30	28.4(5.1)	27.49(4.9)	21.67(0.4)	27.19(0..6)	23.4(1.2)	26.54(0.)	27.72(0.9)	33.84(1.7)
40	30.68(2.4)	32.29(0.8)	27.16(0.6)	28.98(0.4)	29.18(1.7)	33.91(0.9)	34.79(1.3)	38.88(0.9)
60	38.59(0.8)	43.07(0.4)	31.6(1.1)	32.91(0.9)	41.21(1.3)	41.51(0.7)	44.69(2.1)	47.83(3.1)

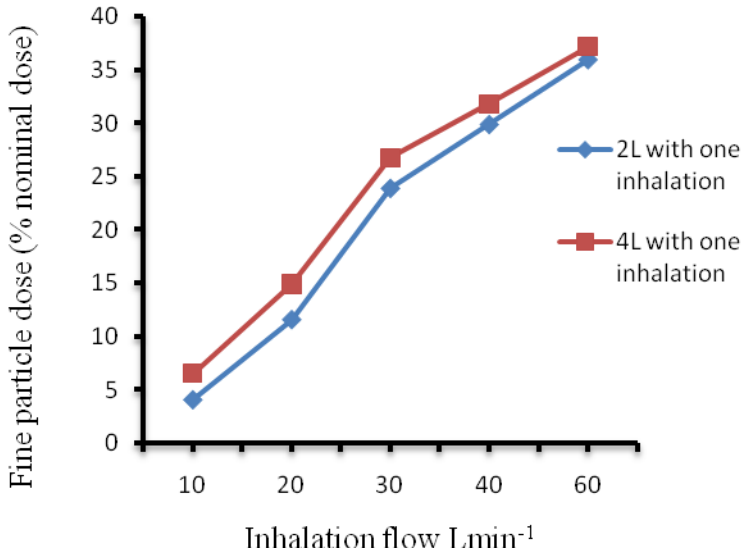


Figure 5.18 Mean fine particle dose (% nominal dose) of salbutamol sulphate from the Accuhaler® at different inhalation flows (10-60) $Lmin^{-1}$ (n=5)

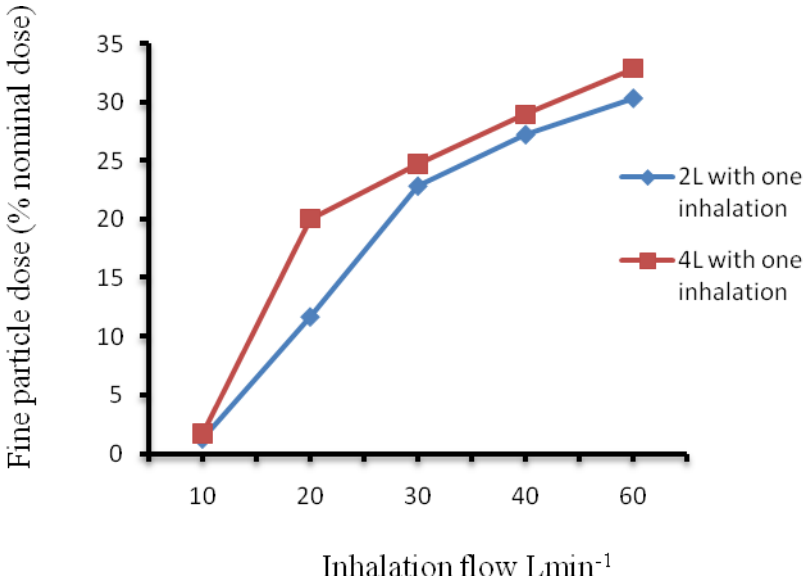


Figure 5.19 Mean fine particle dose (% nominal dose) of salbutamol sulphate from the Easyhaler® at different inhalation flow (10-60) $Lmin^{-1}$ (n=5)

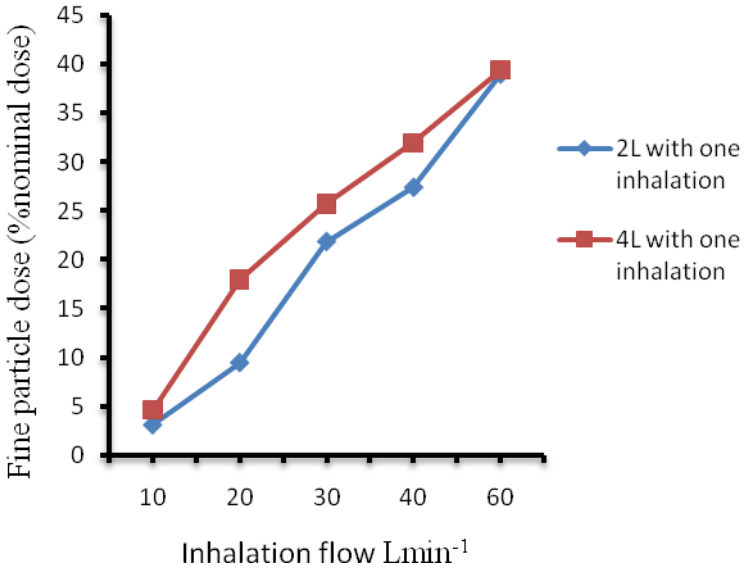


Figure 5.20 Mean fine particle dose (% nominal dose) of salbutamol sulphate from the Clickhaler® at different inhalation flow (10-60) $Lmin^{-1}$ (n=5)

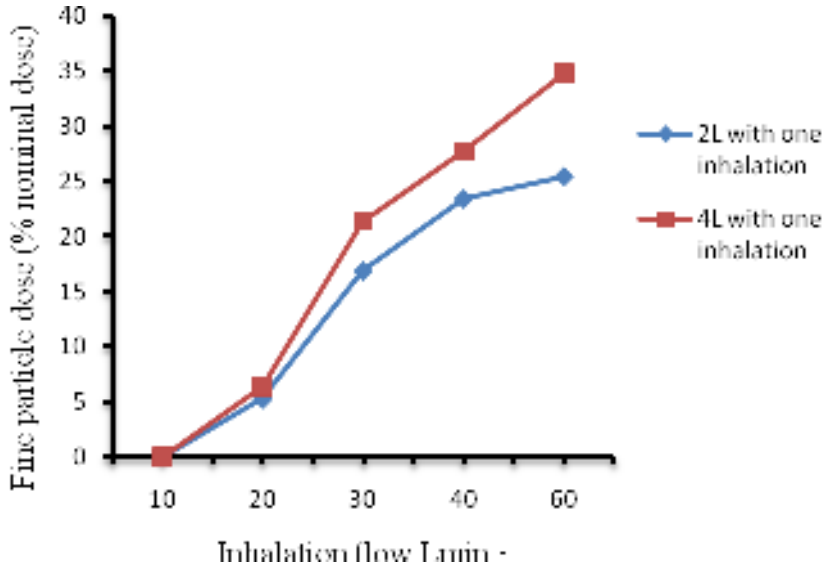


Figure 5.21 Mean fine particle dose (% nominal dose) of salbutamol sulphate from the Turbuhaler® at different inhalation flow (10-60) $Lmin^{-1}$ (n=5)

Table 5.19 Statistical comparison of the fine particle dose (% nominal dose) from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ using a 2L inhaled volume

Inhalation flow	**Mean difference (95 % confidence interval)**			
($Lmin^{-1}$)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10 vs 20	-7.50***(-10.16, -4.85)	10.43***(-11.95, -8.90)	-6.36***(-8.19, -4.53)	-12.74***(-13.95, -11.53)
10 vs 30	-24.35***(-31.93, -16.98)	21.64***(9-25.01, -18.27)	-20.1***(-21.84, -18.17)	-16.81***(-18.19, -15.43)
10 vs 40	-23.59***(-29.92, -23.37)	-25.97***(-27.88, -24.07)	-24.32***(-26.15, -22.50)	21.04***(-22.79, -19.29)
10 vs 60	-31.86***(-34.53, -29.19)	-29.07**(30.29, -27.85)	-38.14***(-40.47, -35.80)	30.09**(-34.98, 25.21)
20 vs 30	-16.85***(-22.93, -10.77)	11.21***(-14.87, -7.54)	-13.65***(-15.08, -11.82)	4.07**(-6.11, -2.03)
20 vs 40	-19.09***(-22.52, -15.66)	-15.54***(-18.92, -12.17)	-17.97***(-20.61, -15.32)	-8.30***(-9.46, -7.15)
20 vs 60	-24.36***(-25.73, -22.99)	18.64***(-19.78, -17.50)	-31.78***(-34.34, -29.23)	17.35***(-21.50, -13.21)
30 vs 40	-2.24(-7.43, 2.94)	4.33***(-8.48, -0.19)	-4.32***(-7.13, -1.51)	-4.23**(-6.99, -1.47)
30 vs 60	-7.51*(-12.55, -2.47)	7.43***(-10.41, -4.44)	-18.13***(-20.88, -15.39)	-13.29***(-19.27, -7.30)
40 vs 60	-5.23*(-7.87, -2.65)	3.09**(-5.99, -0.20)	-13.82**(-14.81, 12.83)	-9.05***(-12.40, -5.70)

The mean difference is significant *P<0.05; **P<0.01; ***P<0.001

Table 5.20 Statistical comparison of the fine particle dose (% nominal dose) from the four different dry powder inhalers at different inhalation flow (10-60) $Lmin^{-1}$ using a 4L inhaled volume

Inhalation flow	**Mean difference (95% confidence interval)**			
($Lmin^{-1}$)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10 vs 20	-8.37***(-9.82, -6.92)	-18.34***(-20.64, -16.04)	-9.83***(-11.47, -8.14)	-11.57*(-13.24, -9.89)
10 vs30	-20.95***(-27.49, -14.41)	-23.03***(-24.08, -21.97)	-17.79***(-20.44, -15.33)	-18.11*(-19.43, -16.78)
10 vs 40	-25.26***(-28.32, -22.29)	-28.16***(-32.01, -24.31)	-24.01***(-27.44, -20.63)	20.16*(-23.64, -16.68)
10 vs60	30.64***(-33.43, -27.86)	-31.63***(-32.88, 30.38)	-33.56***(-36.28, -30.83)	33.65*(-36.07, -31.23)
20 vs 30	-12.58***(-19.62, -5.54)	-4.69***(-7.09, -2.28)	-7.98***(-10.23, -5.60)	-6.54*(-7.61, -5.47)
20 vs 40	-16.89**(-19.39, -14.38)	9.82***(-14.17, -5.46)	-14.18***(-16.71, 11.66)	-8.59*(-12.93, -4.25)
20 vs 60	-22.27***(-25.36, -19.19)	-5.13***(-9.18, -1.08)	-6.22***(-8.26, -4.18)	-22.08***(-24.36, -19.83)
30 vs 40	-4.31(-12.03, 3.41)	13.29***(-15.38, -11.20)	-23.73*(-25.11,-22.34)	2.05(-5.75, 1.64)
30 vs 60	-9.70***(-17.34, -2.06)	-8.60***(-10.65, -6.55)	-15.77***(-17.96, -13.59)	-15.54*(-18.74, 12.38)
40 vs 60	-5.39**(-7.72, -3.06)	-3.47*(-8.11, 1.18)	-9.55***(-12.10, -6.99)	13.48*(-16.88, -10.10)

The mean difference is significant *P<0.05; **P<0.01; ***P<0.001

Table 5.21 Mean (SD) mass median aerodynamic diameter (MMAD), in µm, for the four different dry powder inhalers following one and two inhalations per metered dose for a 2L and a 4L inhalation volumes

	Mean (SD) mass median aerodynamic diameter (MMAD) µm following one inhalation							
Device	Accuhaler		Easyhaler		Clickhaler		Turbuhaler	
Inhalation volume	2L	4L	2L	4L	2L	4L	2L	4L
Inhalation flow (Lmin-1)								
10	6.4(0.3)	6.4(0.3)	6.3(0.5)	6.7(0.3)	5.3(0.5)	7.6(1.3)	7.9(0.7)	7.4(0.2)
20	4.3(0.)	4.5(0.3)	4.2(0.3)	2.3(0.4)	4.8(0.12)	4.7(0.1)	4.0(0.1)	4.(0.1)
30	3.5(0.1)	3.5(0.1)	2.3(0.1)	2.3(0.1)	3.8(0.2)	3.8(0.1)	3.8(0.2)	2.7(0.1)
40	3.1(0.1)	3.1(0.1)	2.2(0)	2.2(0.1)	3.3(0.1)	3.4(0.1)	3.5(0.1)	2.3(0.1)
60	2.5(0)	2.4(0)	2.3(0.5)	2.3((0)	2.6(0.1)	2.6(0)	2.2(0.1)	2.3(0.1)
	Mean (SD) mass median aerodynamic diameter (MMAD µm following two inhalations							
Device	Accuhaler		Easyhaler		Clickhaler		Turbuhaler	
Inhalation volume	2L	4L	2L	4L	2L	4L	2L	4L
Inhalation flow (Lmin-1)								
10	7.9(0.2)	6.0(1.0)	7.7(0.4)	6.7(0.2)	6.5(0.4)	6.8(0.1)	83(0.7)	7.8(0.8)
20	4.2(0)	4.6(0.2)	4.2(0.2)	4.2(0.2)	4.8(0.1)	4.7(0.1)	5.1(0.2)	5.2)
30	3.9(0.1)	3.5(0)	3.3(0.4)	3.3(0.4)	3.9(0.1)	3.7(0.1)	4.1(0.1)	3.7(0.2)
40	3.2(0.1)	3.1(0.1)	3.0(0.1)	3.0(0.1)	3.3(0.1)	3.4(0.)	3.2(0.2)	3.2(0.1)
60	2.5(0)	2.4(0)	2.4(0)	2.4(0)	2.7(0)	2.7(0)	2.8(0.2)	2.8(0)

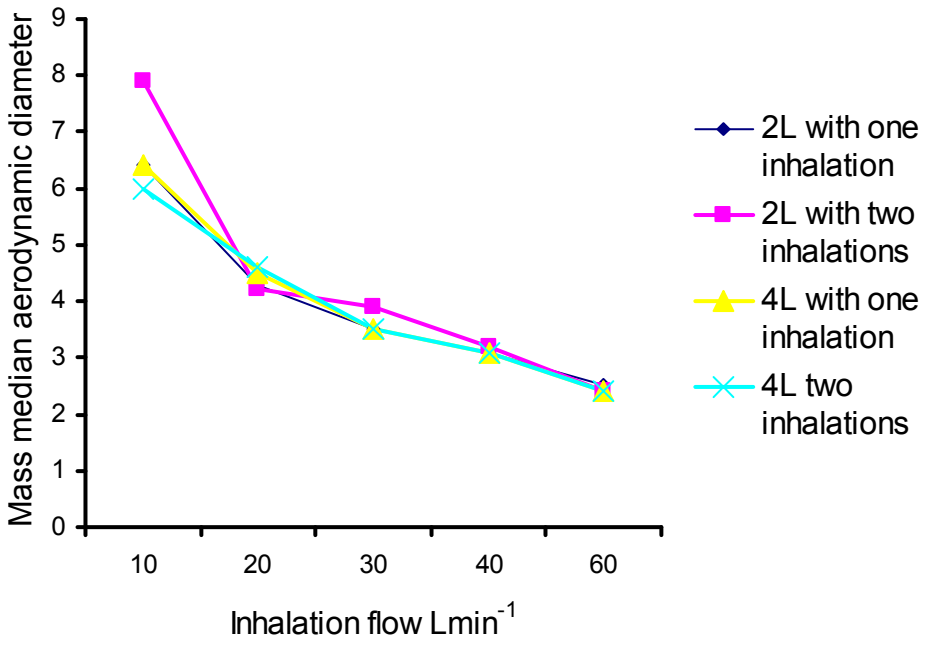


Figure 5.22 Mass median aerodynamic diameter (MMAD in µm) of salbutamol particles from the Accuhaler® at different inhalation flows (10-60) $Lmin^{-1}$

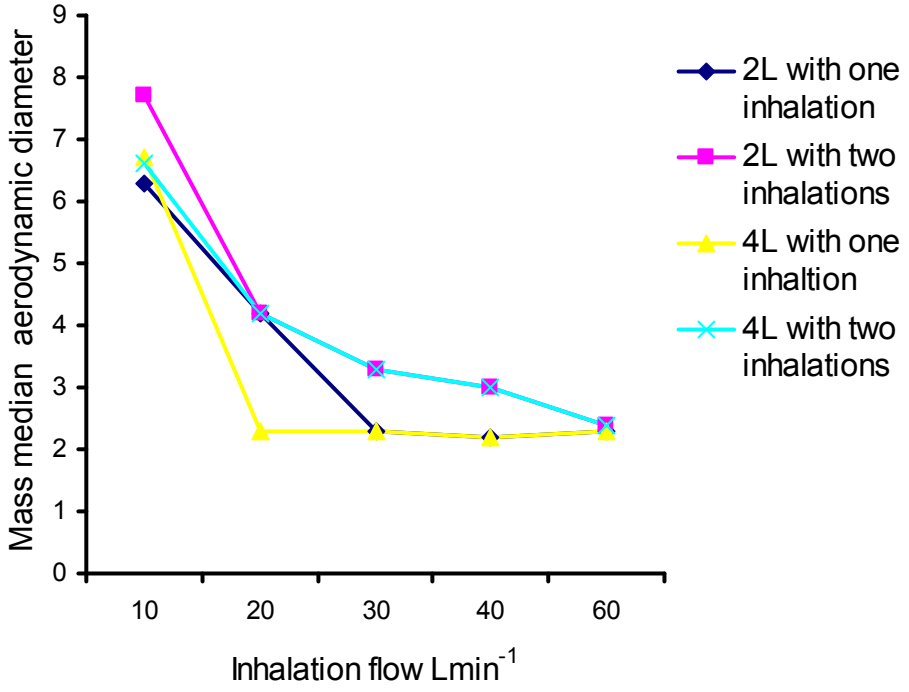


Figure 5.23 Mass median aerodynamic diameter (MMAD in µm) of salbutamol particles from the Easyhaler® at different inhalation flows (10-60) $Lmin^{-1}$

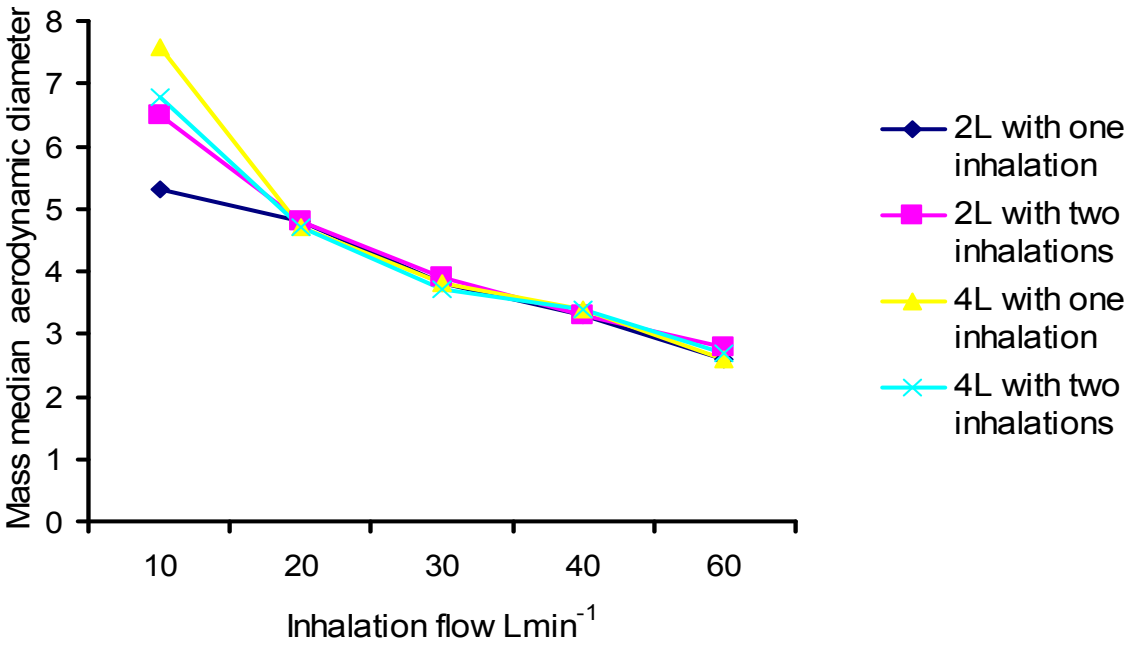


Figure 5.24 Mass median aerodynamic diameter (MMAD in µm) of salbutamol particles from the Clickhaler® at different inhalation flows (10-60) $Lmin^{-1}$

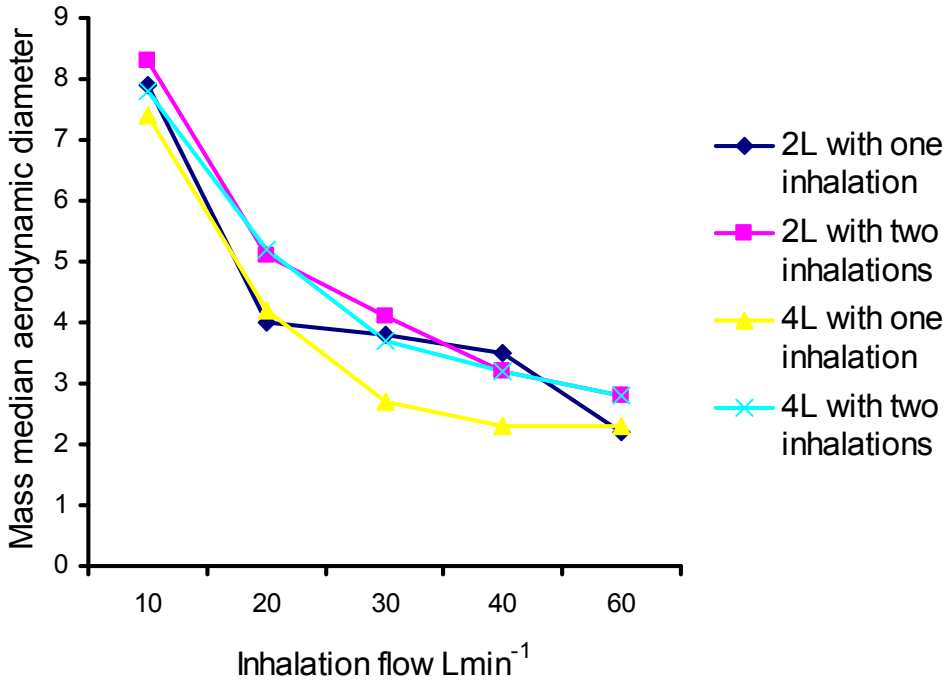


Figure 5.25 Mass median aerodynamic diameter (MMAD in µm) of terbutaline particles from the Turbuhaler® at different inhalation flows (10-60) $Lmin^{-1}$

Figure 5.26 shows the fine particle dose at each inhalation flow with one inhalation per metered dose for a 2L and a 4L inhaled volumes while Table 5.22 presents the statistical comparison of the fine particle dose between the 2L inhaled volume and the 4L inhaled volume. This enabled the evaluation of the effect of the inhalation volume on the fine particle dose from the inhalers under the same inhalation flow.

Figures 5.27 and 5.28 describe the differences in the fine particle dose from the Accuhaler®, Easyhaler®, Clickhaler® and Turbuhaler® between one inhalation and two inhalations at each inhalation flow for 2L and 4L inhaled volumes. There are marginal differences in the fine particle dose emitted from the four inhalers. Tables 5.23 and 5.24 show the statistical comparison of the fine particle dose (% nominal dose) from the four dry powder inhalers between one and two inhalations per metered dose at each inhalation flow using 2L and 4L inhaled volumes. This enabled the evaluation of the effect of the number of inhalations per metered dose on the fine particle dose from the studied inhalers.

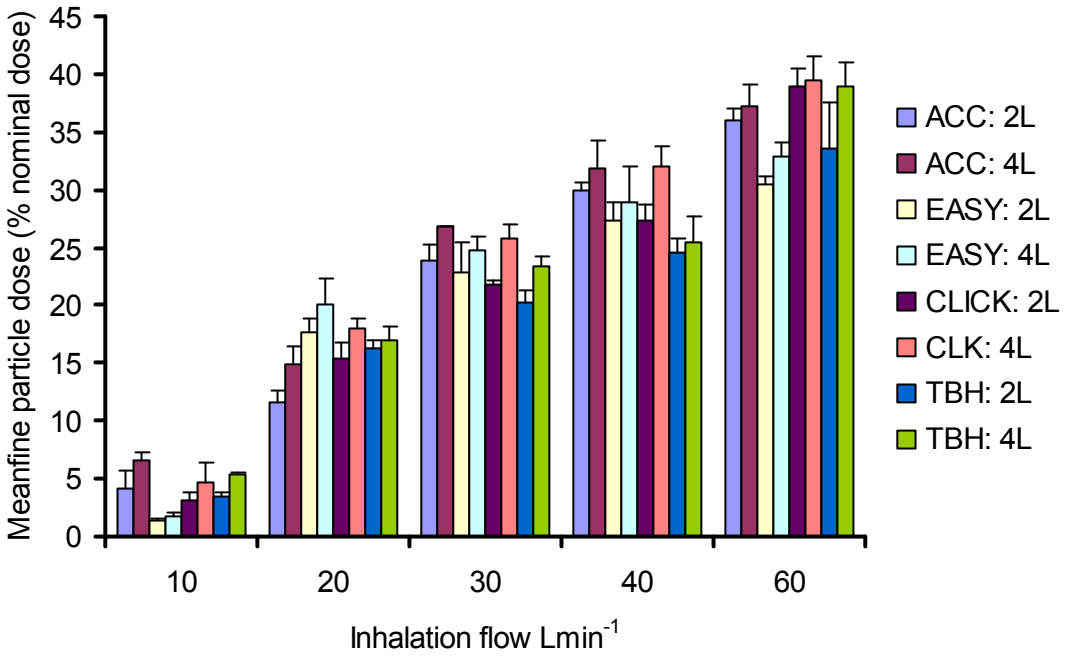


Figure 5.26 Mean (SD) fine particle dose, as % nominal dose from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ following one inhalation per metered dose for 2L and 4L inhalation volumes

Table 5.22 Statistical comparison of the fine particle dose (% nominal dose) from the four different dry powder inhalers between 2L and 4L inhaled volumes each inhalation flow

Inhalation flow ($Lmin^{-1}$)	**Mean difference (95 % confidence interval) (2L vs. 4L)**			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10	-2.46(-5.71, 0.17)	-04.42(-1.24, -0.41)	-4.88*(-7.79, -1.97)	1.85*(-2.35, 1.57)
20	3.32*(-5.71, 0.93)	-8.32*(10.09, -6.57)	-8.35*)-10.78, -5.93)	-0.68(-2.42, 1.05)
30	0.95(-9.78, 11.68)	1.80(-5.46, 1.84)	-2.66*(-3.39, -1.32)	3.16*(-5.38,-0.93)
40	-1.11(-5.64, 3.41)	-2.60(-6.66, 1.45)	-4.56*(-7.67, -1.60)	-0.98(0.54, 3.48)
60	-1.24(-4.37, 1.96)	-2.98*(-3.71, -2.24)	-0.30(-2.07, 1.47)	-5.41(-12.43, 1.60)

*The mean difference is significant *(P<0.05); ** (P<0.01); *** (P<0.001)

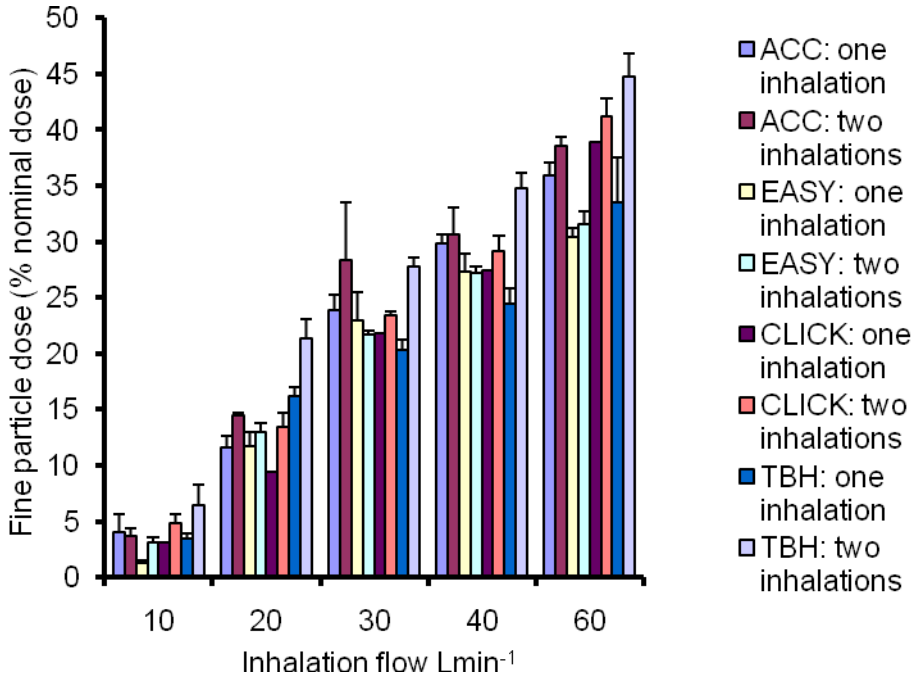


Figure 5.27 Mean (SD) fine particle dose, as % nominal dose, from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ following one and two inhalations per metered dose for a 2L inhaled volume

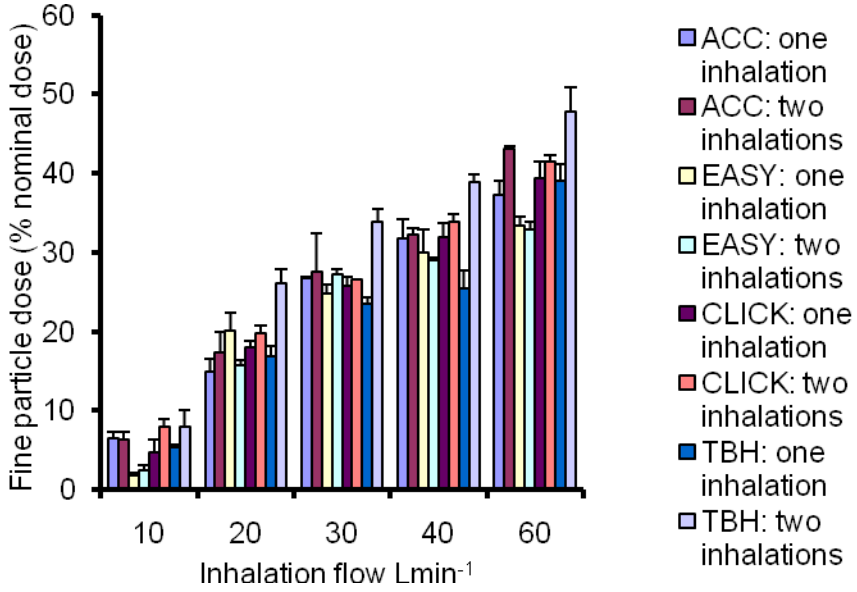


Figure 5.28 Mean (SD) fine particle dose, as % nominal dose, from the four different dry powder inhalers at different inhalation flows (10-60) $Lmin^{-1}$ following one and two inhalations per metered dose for a 4L inhaled volume

Table 5.23 Statistical comparison of the fine particle dose (% nominal dose) from the four different dry powder inhalers between one and two inhalations per metered dose at each inhalation flow using 2L inhaled volume

Inhalation flow ($Lmin^{-1}$)	**Mean difference (95 % confidence interval) (one vs. two inhalations)**			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10	0.23(-1.46, 1.93)	-1.78*(-2.17, -1.39)	-1.75*(-3.16, 0.33)	-2.94*(-5.17, 0.70)
20	2.86*(-4.49, -1.24)	-1.19(-3.02, 0.64)	-1.65(-4.01, 0.72)	-5.10*(-7.82, -2.38)
30	4.55(-2.80, 11.90)	1.34(-1.51, 4.21)	1.24(-0.4, 2.87)	-7.45*(-8.90, -5.97)
40	0.81(-2.09, 3.71)	1.28(-1.60, 1.86)	1.78(-4.55. 0.99)	10.29*(-11.91, -8.67)
60	-2.65*(-3.76, -1.53)	-1.25(-3.13, 0.62)	2.26*(1.36, 3.17)	-11.13(-16.16, -.613)

*The mean difference is significant *(P<0.05); ** (P<0.01); ***(P<0.001

Table 5.24 Statistical comparison of the fine particle dose (% nominal dose) from the four different dry powder inhalers between one and two inhalations per metered dose at each inhalation flow using 4L inhaled volume

Inhalation flow ($Lmin^{-1}$)	**Mean difference (% 95 confidence interval) (one vs. two inhalations)**			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
10	0..25(-1.64, 2.13)	0.66(-1.46, 0.15)	3.31*(1.31, 5.31)	-2.13(4.48, 0.23)
20	-2.37(-6.02, 1.28)	-4.35*(-6.89, -1.80)	-1.97*(-3.40, 0.54)	-9.20(-11.82, -6.54)
30	-0.73(-4.98, 6.45)	-2.46*(-4.07, 0.73)	-0.79(-2.35, 0.75)	10.41*(-12.95, -7.86)
40	0.49(-4.27, 3.29)	0.92(-3.09, 4.93)	-1.95(-4.76, 0.87)	-13.40*(-16.49, -10.30)
60	-5.88*(-7.85, -3.92)	0.45(-1.99, 2.90)	2.09(0.21, 4.39)	-8.86(-13.92, -3.80)

*The mean difference is significant *(P<0.05); ** (P<0.01); *** (P<0.001

5.4 Discussion

In-vitro characterisation of the aerosol is useful as a pre-clinical tool to predict the potential of the emitted dose to deposit into the lungs. In this study, the results indicated bimodal mass size distribution of the dose emitted from the Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler® upon inhalation across the inhalation flow range of 10 to 60 $Lmin^{-1}$. At each inhalation flow, over 50% of the mass contains large particles. These particles have an aerodynamic diameter greater than 5µm and are deposited by (inertial impaction) on the upper stages (induction port, mixing inlet, and preseparator) of the impactor (Hillery et al., 2001). These stages of the impactor morphologically correspond to the oropharyngeal region (Weibel 1963; Hickey 1992). Particles with an aerodynamic diameter smaller than 5µm were deposited on the lower stages and filter. The particle size distribution on the upper stages shows that deposition in the induction port decreased while that in the preseparator increased as the inhalation flow (hence turbulent energy for particle de-aggregation) increased for the four inhalers. Thus more proportion of the smaller particles penetrated into the lower stages of the impactor (Figures 5.2 to 5.17).

Figures 5.18 to 5.21 show the influence of inhalation flow on the fine particle dose (FPD). The FPD (containing drug particles with an aerodynamic diameter ≤ 5µm) increased with an increase in the inhalation flow upon inhalation. An explanation for this observed trend can be found in the study reported by (Clark and Hollingworth 1993).The study highlighted that the turbulent energy inside a DPI is represented by a pressure change ($\sqrt{\Delta P}$) that is generated inside the device during an inhalation. This pressure change is directly related to the DPI's internal resistance to airflow (R) and the inhalation flow (Q) and the relationship is described as: $\sqrt{\Delta P}$ = QR. Since the turbulent energy required inside a DPI to transform the metered powder formulation into an emitted dose containing fine particles with the potential for lung deposition (a respirable dose) is a product of the inhalation flow and the inhaler's internal resistance, then the faster the flow then the higher

will be the energy. Therefore the higher energy results to greater de-aggregation of the metered powder formulation into a fine particle dose. Hence, the better is the quality of the emitted dose for lung deposition. Figures 5.18 to 5.21 show the variability of the FPD from the four different inhalers with the inhalation flow. The change in the mean FPD (% nominal dose) with the inhalation flow from 10 to 60 $Lmin^{-1}$ following one inhalation per metered dose are in the ranges of: 4.1 to 37.19, 1.4 to 33.4, 3.7 to 39.42 and 3.4 to 38.97 for the salbutamol-containing Accuhaler®, Easyhaler®, and Clickhaler® and for the terbutaline Turbuhaler® respectively (Table 5.18). Similar data were respectively obtained for the inhalers following two inhalations (Table 5.18). Two inhalations per dose are therefore not required. The Accuhaler® and Easyhaler® have lower range than the Clickhaler® and the Turbuhaler®. At an inhalation flow below 30 $Lmin^{-1}$ FPD emitted from the and the Easyhaler® declined sharply, while the variation of FPD was relatively constant at the flow range of 30-60 $Lmin^{-1}$ (Figure 5.18 and 5.19). For the Clickhaler® and the Turbuhaler® the trends showed a pronounced variation on FPD an increase of the inhalation flow from 10 to 60 $Lmin^{-1}$. The difference in behaviour may be attributed to differences in formulations designs. Factors such as the internal structure of each device as well as the drug to excipient ratio may account for the varying resistance to inhalation flow, hence the amount of fine particle dose emitted from these inhalers.

Statistical comparison of the differences in the FPD between inhalation flows presented in Tables 5.19 and 5.20 confirmed that the FPD significantly ($p<0.001$) increased with the increase in the inhalation flow. Overall, the results of this study have demonstrated the flow dependent dose emission property of the four studied inhalers, with the salbutamol Accuhaler® and Easyhaler® less affected compared with the salbutamol Clickhaler® and the terbutaline Turbuhaler. Also the finding is in agreement with the previous reported in-vitro studies on the flow dependent FPD characteristics of salbutamol Accuhaler® greater

and Easyhale®r (Palander et al. 2000), terbutaline Turbuhaler (Malton et al, 1996; Ross and Schulz, 1996).

The mass median aerodynamic diameter (MMAD) provides an indication of size distribution of drug particles deposited in the lower stages of the impactor (and hence the likely drug deposition site in vivo). The results show that the MMAD decreased with an increase in the inhalation flow for the studied inhalers (Figures 5.22 to 5.25). The explanation of this trend can be found in the fact that the faster the inhalation flow through a DPI (hence energy), the greater is the force inside the device to de-aggregate the drug-carrier complex or pure pellets into fine particles with a reduced aerodynamic diameter thereby enhancing penetration to the lower stages of the impactor. These lower stages are surrogates of the lower regions of the human respiratory system (Weibel 1963; Hickey 1992).

For each DPI, there will be a minimum inhalation flow (hence threshold energy) for the dose emission with sufficient potential for lung deposition when patients inhale fast as they can. This value may differ due to differences in the design-formulation combination. The data presented in Tables (5.2 to 5.17) show that the minimum inhalation flow for the dose emission (FPD) with sufficient potential for lung deposition for the Accuhaler®, Easyhaler® and Clickhaler® is 20 $Lmin^{-1}$, while that for the Turbuhaler® is about 30 $Lmin^{-1}$. Although the in-vitro data obtained should be viewed with caution as the data obtained from cascade impaction do not always reflect the clinical situation, they can be used to guide clinical response (Barry and O`Callaghan, 2003).

The inhalation flow through a DPI is affected by its intrinsic resistance. Studies have shown (Tarsin, et al, 2000) that the Accuhaler® has low resistance, whereas the Clickhaler®, Turbuhaler® and the Easyhaler® all have high resistance. Patients with COPD have been reported to have lower inhalation flows than adult asthmatics. Also the more severe the obstruction of the airways the lower were the inhalation flows through a

variety of inhalers (Tarsin et al. 2001). Thus, the efficacy of an inhaled product will be affected by the age and abilities of the patient who is asked to use it.

Some asthmatic children and COPD patients were unable to generate the minimum inhalation flow of 30 $Lmin^{-1}$ through a Turbuhaler® (Pedersen et al. 1990; Broeder et al. 2003). The finding about the terbutaline Turbuhaler® in this study implies that some patients with asthma//COPD may not be able generate a dose with the potential for lung deposition from the Turbuhaler® to achieve control. Thus, once their inhalation technique is identified with the aid of the In-Check Dial®, they should be given intensive training if they still prefer the Turbuhaler® or an alternative that suits their natural technique should be prescribed.

The Accuhaler has low resistance to flow and thus less effort is required to achieve any given inspiratory flow. Conway et al. (1996) reported that the mean inspiratory flow of 55 patients with asthma (aged 5 to 50) through the Accuhaler® was about $117Lmin^{-1}$. Therefore, the minimum inhalation flow of 20 $Lmin^{-1}$ through the Accuhaler® to emit a dose with sufficient potential for lung deposition obtained in this study seems easily achievable. Hence, the force of inhalation may not be a critical factor when training patients to correctly use the Accuhaler®. Although the Easyhaler® has high resistance, a study comparing the bronchodilating effect of salbutamol (100µg) delivered via Easyhaler® at low inspiratory flow (as low as 16 Lmin-1) showed that the Easyhaler® produced an equivalent improvement in lung function to a correctly used metered dose inhaler plus spacer (Koskela et al. 2000). Similar clinical studies with asthmatic children using a Clickhaler® (intermediate resistance inhaler) at inspiratory flow of 15, 30 and 60 $Lmin^{-1}$ have demonstrated mean improvements in FEV_1 of 0.44, 0.45, and 0.53 L respectively. The studies highlighted that clinical efficacy of the Clickhaler® is independent of inspiratory flows in the range of (15 – 60) $Lmin^{-1}$ (Newhouse et al. 1999). In this study the minimum flow for potential lung deposition with the Easyhaler® and the

Clickhaler® has been identified to be 20 $Lmin^{-1}$. Thus, the Easyhaler® and the Clickhaler® can be used with confidence in patients who may have difficulty in generating a high inspiratory flow such as children and the elderly.

In order to test the effect of inhalation volume on the FPD generation from the DPIs, the data obtained from a 2L volume was compared with a 4L volume at each inhalation flow as shown in Figure 5.26. The FPD (% nominal dose) at 2L and 4L emitted from the Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler® were similar at inhalation flow range of 10 to 60 $Lmin^{-1}$ (Table 5.18). In general, at each inhalation flow a higher FPD was emitted from the four inhalers with a 4L inhaled volume than a 2L. However, these differences were not statistically significant in most cases as shown in Table 5.22. Although it was expected that a higher FPD may be obtained with a larger inhaled volume (because of the greater energy input), no significant difference was observed in this study. Also interesting there were no differences in MMAD at each inhalation flow for a 2L inhaled volume compared with a 4L inhaled volume. Thus, particle distribution was not affected by inhalation volume with the four studied inhalers.

The explanation to this observation can be found in the reported studies by De Boer et al, (1997) and Everard et al. (1997). These researchers highlighted that the device-formulation combination together with the rate of increase in the inhalation flow (acceleration rate) is a very important factor in the generation of the fine particle dose from a DPI. This acceleration rate correlates to the peak inhalation flow achieved by patients (Broeders et al. 2001). The steeper is the increase (exerts a greater air-impact on powder formulation), the more is the fine particle dose generated inside the DPI during an inhalation manoeuvre. However, the turbulent energy inside a DPI is a product of the patient's inhalation flow and the resistance inside the inhaler. Therefore, the rate at which energy is applied to particles, and not just the total amount of energy, influences de-aggregation to fine particles. They concluded that dose emission from DPIs formulated

with either a reservoir or blister occurs immediately at the start of the inhalation, that is a short and fast inspiration through a DPI gives an optimal fine particle output. Therefore, for the Accuhaler (a multidose strip-type) and the multidose/reservoir-Easyhaler, Clickhaler and Turbuhaler at any given inhalation flow the energy impacted on the powder to overcome attractive forces between particles, or particle and carrier, by a 2L inhaled volume may not be significantly different from that provided by a 4L inhaled volume. Hence, the effect of inhalation volume on the emitted dose (fine particle dose) when patients inhale `as fast as they can` through the multidose DPIs (either a reservoir or a strip) is negligible. The finding about the effect of inhalation volume on the FPD from these multidose DPIs in this chapter concurred with that on the total emitted dose in chapter 4 and similar previous in-vitro study using a Turbuhaler® (multidose/reservoir inhaler) and a Diskhaler® (multidose/strip inhaler) by the De Boer et al, (1997).

The effect of the number of inhalation on the fine particle dose (FPD) from the Accuhaler, Easyhaler®, Clickhaler® and the Turbuhaler® at different inhalation flows (10 to 60 $Lmin^{-1}$) using the same inhalation volume is shown in Figures 5.27 and 5.28 (for 2L and 4 L inhalation volumes). In general, the (FPD) from the four studied inhalers is slightly greater with two inhalations than one inhalation at each inhalation for a 2L inhaled volume and a 4L inhaled volume respectively. However, these differences were not statistically significant ($p<0.05$) in most cases except for the Turbuhaler at low inhalation flow (range 10-40 $Lmin^{-1}$) using a 2L inhaled volume (Table 5.22). These may be considered as isolated cases that may not be of any clinical relevance. Overall, the influence of the number of inhalations on the fine particle dose hence lung deposition and ultimately clinical effects is negligible.

5.5 Conclusion

In conclusion, this study highlights that the Accuhaler, Easyhaler, Clickhaler, and the Turbuhaler all showed flow-dependent fine particle dose emission characteristics to a

varying extent. Also the study has identified that the minimum inhalation flow for the dose emission with sufficient potential for lung deposition when patients inhale as fast as they can for the Accuhaler, ® Easyhaler® and the Clickhaler® is about 20 $Lmin^{-1}$ while that for the Turbuhaler® is about 30 $Lmin^{-1}$. The finding, though in-vitro, relates to the reported clinical studies. These values together with the use of the In-Check dial provide insights into the choice of an inhaler that suits a patient's natural technique for effective lung deposition.

Furthermore, the four studied inhalers showed insignificant difference in the total fine particle dose between a 2L inhalation volume and a 4L inhalation volume at each inhalation flows. This study demonstrates that either a 2L or a 4L inhalation volume can be used for the in-vitro measurement of aerodynamic dose emission characteristics from multidose DPIs (either reservoir or strip-type). Since asthmatic patients have an average inhalation volume of about 2L when they inhaled through a DPI and they are the ultimate users of these inhalers, a 2L inhaled volume rather than the compendial recommended 4L inhaled volume should be considered for in-vitro tests.

Although the Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler® generally showed a significantly greater total emitted dose with two inhalations than one inhalation per metered dose across the inhalation flows (range 10-60 $Lmin^{-1}$) as reported in chapter 4, there were no statistically significant differences in their fine particle dose between one and two inhalations. Since it is the fine particle dose that determines the lung deposition and ultimately the clinical effects, the finding in this study tends to support the continuation of the present manufacturers` instructions for patients to inhale once for each metered dose `as fast as they can`.

Chapter 6

6 Ex- vivo determination of dose emission from dry powder inhalers (DPIs) at slow and fast inhalation flows using the In-Check® Dial

6.1 Introduction

Lung deposition from an inhaler is dependent on the inhaler, its formulation and the inhalation technique used. Each inhaler due to its formulation and device-design requires a specific inhalation technique. For a DPI the inhalation manoeuvre should be fast from the start of the inhalation and sustained for as long as possible. Many patients experience problems using their devices correctly. Poor inhalation technique can markedly reduce the proportion of the drug that reaches the lungs. Studies suggested that 28-68% of patients with asthma have problems using their MDI or DPI sufficiently well to benefit from the dose (Raul, 2006). Overall, the issue of correct use is of critical importance in maintaining optimal asthma control as patients who misuse their inhalers tend to have less stable asthma than those who use their device correctly (Giraud and Roche, 2002).

The In-Check Dial® has been introduced to identify a patient's inhalation flow through an inhaler and is useful to identify an inhaler to suit a patient's natural technique. The mouthpiece has a dial that can be turned so that it is set to simulate the resistance of several commonly used inhaled products. This is achieved by altering the diameter of the inhalation orifice/hole such that the resistance is the same as that of the selected inhaler. The rate of inhalation is measured by reading the value on the meter. When the patient inhales through the In-Check Dial the reading identifies the inhalation rate that would be obtained when using the inhaler for which the meter has been set for. The In-Check Dial has been externally tested in-vitro by AEA Technology and found to provide similar resistance to the inhalation devices available.

(http://www.clement.clarke.com/inspiratory/In-check).

Studies have highlighted the potential of the In-Check Dial to identify patient inhalation rates and thus inspiratory effort of all type of patients using different dry powder inhalers (Tarsin et al. 2000). Other studies have shown that DPIs operate effectively at peak inhalation flow >30Lmin^{-1} and that the optimum flow for some DPIs in terms of the total emitted dose and the fine particle dose is >60Lmin^{-1} (Bisgaard et al. 1998; Nielsen et al. 1998). Therefore, slow (30 Lmin^{-1}) and fast (60 Lmin^{-1}) inhalation flows have been chosen for this study because they are widely thought to be clinically relevant for patients with asthma (Chrystyn, 2006).

The present study was designed to use an ex - vivo approach with the aid of the In-Check Dial® to determine the dose emission from a variety of multidose DPIs at slow and fast inhalation flows following one and two inhalations per metered dose. The DPIs used were the salbutamol Accuhaler®, Easyhaler® and Clickhaler® and the terbutaline Turbuhaler®. At present the instructions (manufacturer's patient information leaflet) for using these multi-dose DPIs state only one inhalation per dose and patients should inhale as fast as they can. Since dose emission is dependent on inhalation flow, this study has included the ex-vivo dose emission after one and two inhalations per metered dose.

6.2 Method

Instrumentation and devices

The In-Check Dial® (Clement Clarke International, Harlow UK)

Pari electrostatic filter pad (Pari Gmbh, Starnberg, Germany)

Filter holder (Copley Scientific, Nottingham, UK)

Inhaler devices are: Ventolin® Accuhaler® (salbutamol sulphate 200 µg per label dose GlaxoSmithKline, UK); Asmasal® Clickhaler® (salbutamol sulphate 114 µg per label dose ULB, UK); Easyhaler® (salbutamol sulphate 200 µg per label dose); Orion Pharma, Finland) and Bricanyl® Turbuhaler® (Labelled as nominal dose of terbutaline sulphate 500 µg per dose Astra Zeneca, UK).

6.2.1 Procedure

Twelve non-smoking healthy volunteers (six females) have been included in the study. The demographic data of the individuals that participated are as follows:

Volunteer code	Gender	Age	Height	Weight	FEV_1
1	Female	24.0	161.0	62.0	92.0
2	Male	23.0	163.0	50.0	99.0
3	Male	37.0	178.0	70.0	98.0
4	Male	28.0	164.0	59.0	90.0
5	Male	24.0	162.0	54.0	95.0
6	Female	34.0	172.0	69.0	98.0
7	Female	27.0	169.0	57.0	97.0
8	Female	25.0	163.0	70.0	99.0
9	Female	30.0	174.0	68.0	99.0
10	Female	31.0	172.0	62.0	91.0
11	Male	50.0	175.0	67.0	97.0
12	Male	41.0	173.0	72.0	98.0
Mean		**31.2**	**168.8**	**65.6**	**96.1**
SD		**3.6**	**5.1**	**12.7**	**3.7**

The local research ethics panel approval was obtained prior to this study and all volunteers gave signed informed consent. The twelve non-smoking healthy adult volunteers (six females) were trained to inhale through each dry powder inhaler (DPI) using slow ($30Lmin^{-1}$) and fast ($60Lmin^{-1}$) inhalation flows with the In-Check Dial® (Clement Clark, UK) set to correspond with the orifice/resistance of the inhalers under test. Then each volunteer inhaled at `the trained inhalation flows` through an active inhaler (according to the manufacturer's instructions to patients).The mouthpiece of each inhaler was attached to a tightly-fitted mouthpiece adaptor. This contained a filter holder with a Pari® filter pad. This was therefore placed between the mouthpiece of the DPI and the volunteer's mouth as shown in Figure 6.1. The inhaled drug was entrained on the filter. On each occasion each

volunteer first inhaled once from a metered dose. The filter assembly was then replaced by a new one and the volunteer performed two inhalations from another metered dose. The filter assembly was dismantled: the mouthpiece, the filter-holder and the filter were rinsed with bamethane sulphate in distilled water (internal standard) for the HPLC quantitation of the emitted dose. Four separate determinations (n=4) were performed at each inhalation flow by each volunteer.

.The DPIs used were the salbutamol Accuhaler, Clickhaler and Easyhaler and the terbutaline Turbuhaler.

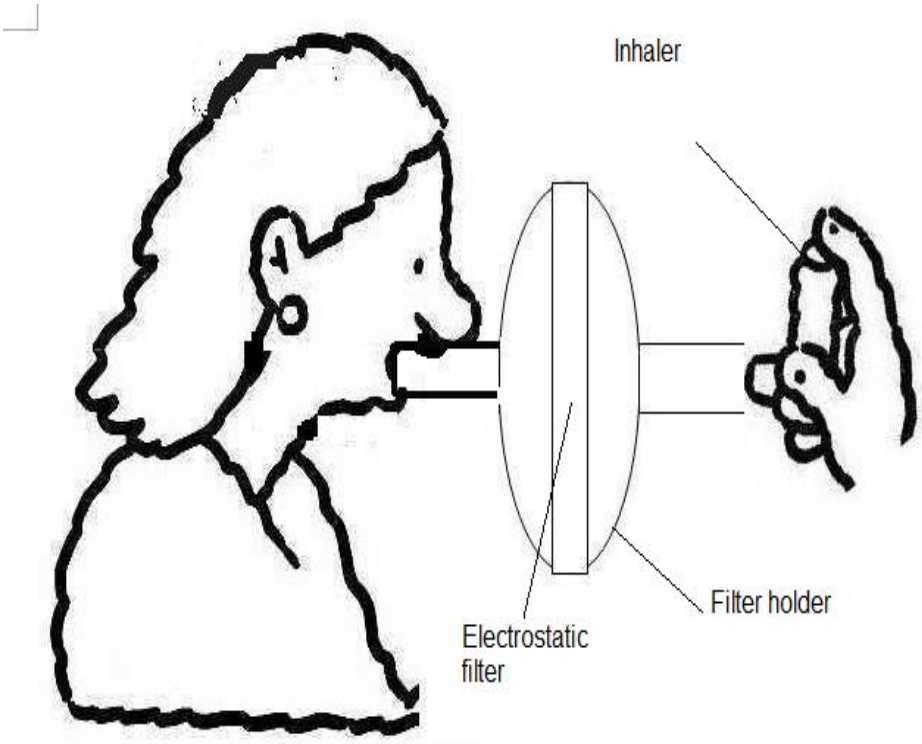


Figure 6.1 A schematic diagram of the ex-vivo determination of the emitted dose

6.2.2 Analysis of data

The emitted dose was calculated as µg per dose. Since the label claims of the studied inhalers differ, the emitted dose from each inhaler was expressed as a percentage of the nominal dose (label claim).

6.2.3 Statistical analysis

SPSS version 15.0 software (SPSS Inc., Chicago, USA) was used for the statistical analysis. A two-way analysis of variance (ANOVA) with the application of the General Linear Model Univariate was used to determine any significant differences in the emitted dose from four different inhalers and the two inhalation flows. Also the statistical comparisons of the emitted dose between one and two inhalations for each metered dose under the same flows were made. The mean difference (95% confidence interval) was calculated and a probability value of ($p<0.05$) was considered being significant.

6.3 Results

The data presented in tables 6.1 to 6.4 describes the total emitted dose from each of the four inhalers at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows. A summary data on the mean (SD) total emitted dose (% nominal dose) from the four inhalers, following one inhalation per metered dose at 30$Lmin^{-1}$and 60$Lmin^{-1}$ inhalation flows from the Accuhaler, the Easyhaler, the Clickhaler and the Turbuhaler respectively were: 80.54(16.5) and 93.10(16.1); 78.6(16.9) and 95.0(15.7); 57.4(12.3) and 73.7(12.7) and 58.7(23.0) and 81.3(16.7). Similarly, the mean (SD) total emitted dose (% nominal dose) from the four inhalers, following two inhalations per metered dose were: 90.6(22) and 102(24.0); 84.8(22.0) and 103(20.0); 67.1(19.1) and 81.7(23.5); and 67.9(18.5) and 91.4(21.8).These summaries are presented in Tables 6.5. Figures 6.2, to 6.5 described variation of the total emitted dose (%nominal dose) from each of the four inhalers with inhalation flows (30 and 60) $Lmin^{-1}$ following one and two inhalations.

The statistical comparisons of the emitted dose (% nominal dose) from the four DPIs between inhalation flows and between one and two inhalations for each dose are presented in Tables 6.6 and 6.7. Figure 6.6 shows the comparison of the mean (SD) total emitted dose between one and two inhalations per metered dose at slow and fast (30 and 60 $Lmin^{-1}$) inhalation flows for the Accuhaler, the Easyhaler, the Clickhaler and the Turbuhaler.

Table 6.1 Total emitted dose of salbutamol sulphate (% nominal dose) from the Accuhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Inhalation flow ($Lmin^{-1}$)	**Total emitted dose (% nominal dose)**			
	slow (30)		Fast (60)	
No. of inhalations	one	two	one	two
Volunteer				
1	88.9	102	97.3	113.1
2	59.6	59.6	66.9	66.9
3	53.1	58.8	58.7	58.7
4	83.4	83.4	96.7	101.2
5	92.6	101.5	108.4	122.6
6	89.5	89.6	107	115.6
7	72.7	73.8	86.4	86.4
8	57.9	85	93.1	93.1
9	87.3	97.9	97.9	110.5
10	107.9	139.6	116	147.7
11	92	105.5	97.1	107.5
12	81.6	91	91.7	101
Mean	**80.5**	**90.6**	**93.1**	**102.03**
SD	**16.5**	**21.9**	**16.3**	**24.0**
RSD	**20.5**	**24.1**	**17.6**	**23.6**

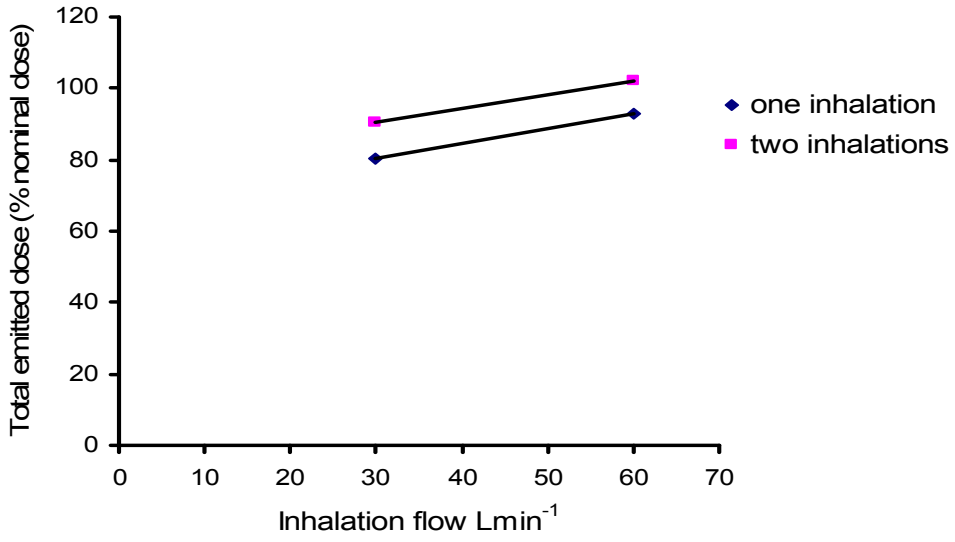


Figure 6.2 Total emitted dose of salbutamol sulphate (% nominal dose) from the Accuhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Table 6.2 Total emitted dose of salbutamol sulphate (%nominal dose) from the Easyhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Inhalation flow ($Lmin^{-1}$)	**Total emitted dose (% nominal dose)**			
	slow (30)		Fast (60)	
No. of inhalations	one	two	one	two
Volunteer				
1	72	80.2	90.9	106.9
2	60	60	74.6	74.6
3	59.3	59.3	94.4	94.4
4	70.4	78.5	84.2	84.2
5	86.6	96.3	95.4	106.7
6	67.9	69.3	88.2	102.1
7	66.9	70.8	88.8	102
8	77	77	78.6	91.2
9	80.8	90.6	127.8	138.2
10	119	138.8	121.7	143.5
11	90.6	103.5	95.8	101.2
12	92.7	92.7	99.2	102.8
Mean	**78.6**	**84.8**	**95.0**	**103.98**
SD	**16.9**	**22.0**	**15.7**	**19.7**
RSD	**21.5**	**25.9**	**16.5**	**19.0**

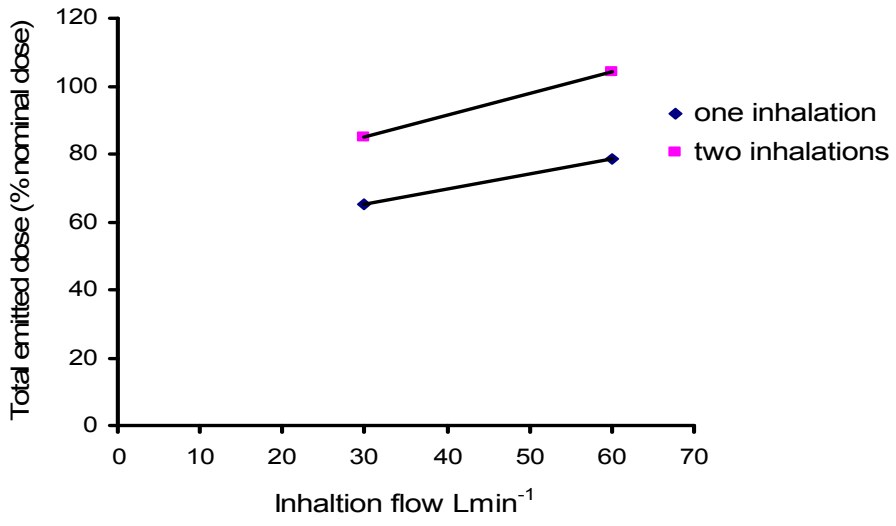


Figure 6.3 Total emitted dose of salbutamol sulphate (% nominal dose) from the Easyhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Table 6.3 Total emitted dose of salbutamol sulphate (% nominal dose) from the Clickhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Inhalation flow ($Lmin^{-1}$)	**Total emitted dose (% nominal dose)**			
	slow (30)		Fast (60)	
No. of inhalations	one	two	one	two
Volunteer				
1	63.9	81.7	71.3	80.8
2	25.9	25.9	51.9	60.9
3	55	56.9	69.8	73.8
4	49.3	49.3	71.5	71.5
5	72.5	80.4	76.2	84.7
6	66.4	74.1	81.5	84.5
7	66.2	72.2	82	82
8	61.14	61.14	64.2	64.2
9	58.7	70.8	67.3	68.3
10	51.4	94.9	105.2	150.4
11	66.5	86	76	91
12	51.5	51.5	68	68
Mean	**57.4**	**67.1**	**73.7**	**81.68**
SD	**12.3**	**19.1**	**12.8**	**23.5**
RSD	**21.4**	**28.5**	**17.3**	**28.8**

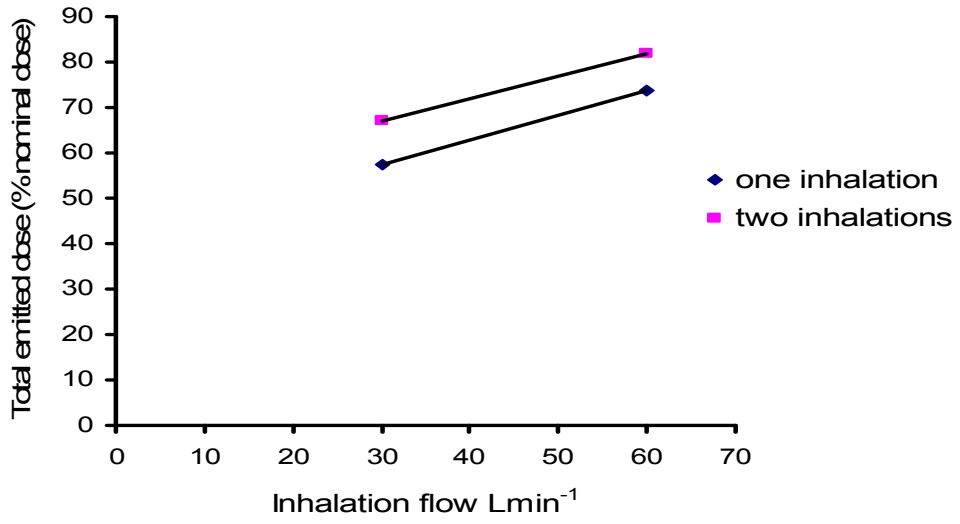


Figure 6.4 Total emitted dose of salbutamol sulphate (% nominal dose) from the Clickhaler® following one and two inhalations at slow (30$Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Table 6.4 Total emitted dose of terbutaline sulphate (% nominal dose) from the Turbuhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Inhalation flow ($Lmin^{-1}$)	**Total emitted dose (% nominal dose)**			
	slow (30)		Fast (60)	
No. of inhalations	one	two	one	two
Volunteer				
1	56.9	62.1	90	97.9
2	3.6	40.1	61.5	71.2
3	43	46.8	76.98	88.88
4	77	90.7	82.4	95.5
5	53	59.4	60	66.8
6	79.6	85.2	105.7	132
7	69.8	69.8	80.3	80.3
8	89.5	90.7	109	127.4
9	49.5	53	56	60
10	64.9	86.3	91.9	100.3
11	75.3	81.4	81.9	89.6
12	42.4	48.9	80.2	87
Mean	**58.7**	**61.9**	**81.3**	**91.4**
SD	**23.0**	**18.5**	**16.7**	**21.8**
RSD	**39.2**	**27.3**	**20.5**	**23.9**

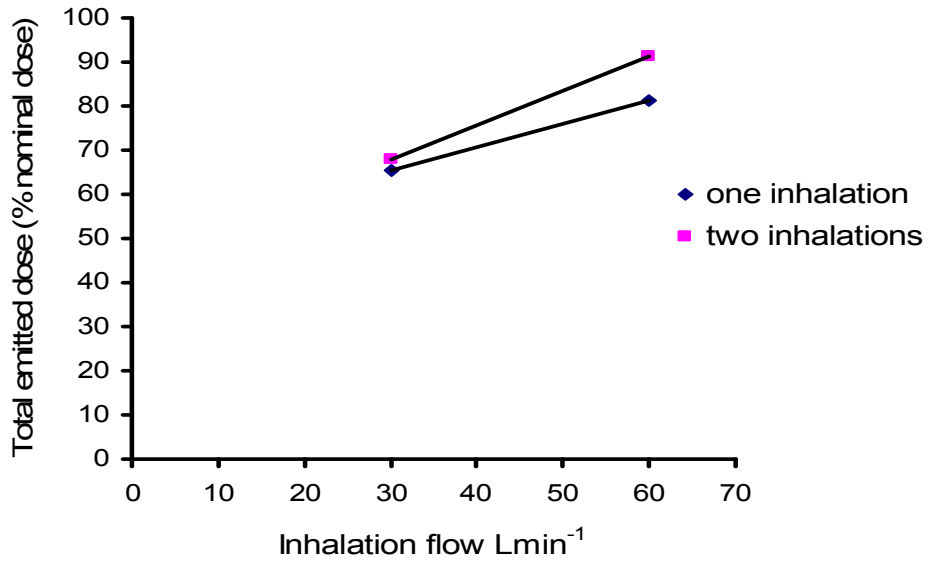


Figure 6.5 Total emitted dose of terbutaline sulphate (% nominal dose) from the Turbuhaler® following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows

Table 6.5 Mean (SD) total emitted dose (% nominal dose) from the four DPIs following one and two inhalations for each dose by the 12 volunteers

Mean (SD) total emitted dose (% nominal dose) for one inhalation				
Inhalation flow ($Lmin^{-1}$)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30	80.5(16.5)	65.3(23.1)	57.4(12.3)	58.71
60	93.1(16.3)	78.6(35.4)	73.7(12.8)	81.3(16.7)
Mean (SD) total emitted dose (% nominal dose) for two inhalations				
Inhalation flow ($Lmin^{-1}$)	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30	90.6(21.9)	84.8(26)	67.1(19.1)	67.1(18.5)
60	102(24)	104(15.2)	81.6(23.5)	91.4(21.8)

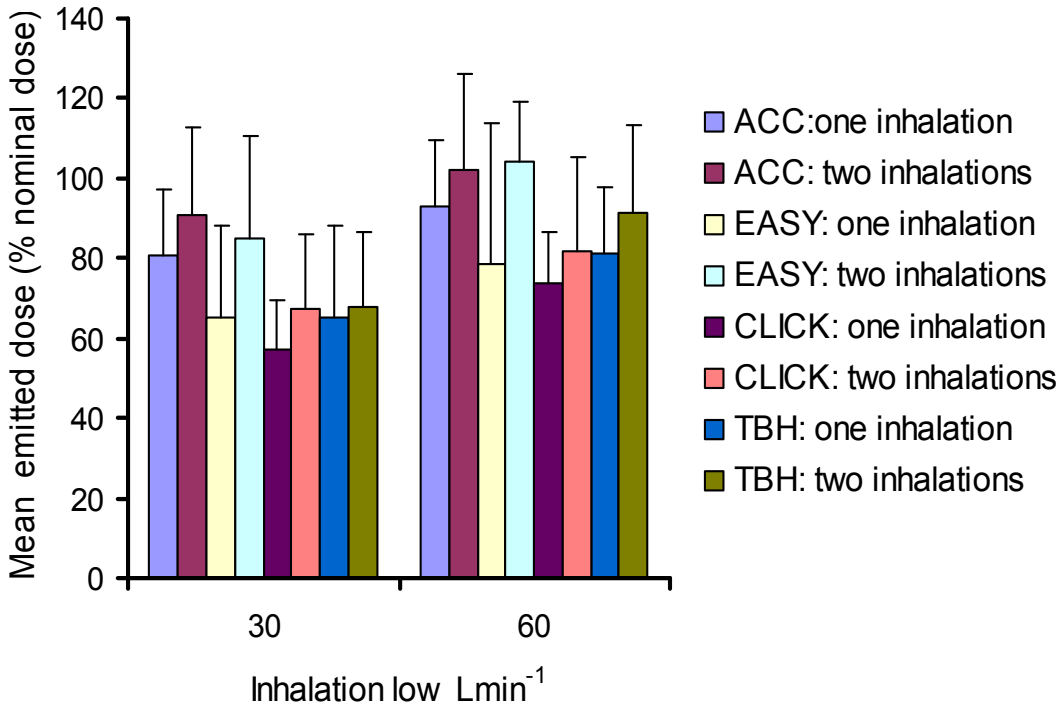


Figure 6.6 Mean (SD) total emitted dose (% nominal dose) from the four DPIs following one and two inhalations at slow (30 $Lmin^{-1}$) and fast (60 $Lmin^{-1}$) inhalation flows by the 12 volunteers

Table 6.6 Statistical comparisons of differences in emitted dose (% nominal dose) from the four DPIs between inhalation flows

Inhalation flow ($Lmin^{-1}$)	Mean difference (95% confidence interval) Following one inhalation			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30 vs. 60	12.56***(-17.72, -7.39)	16.37**(-25.01, -7.72)	-24.31**(-40.1, -8.54)	22.61**(-33.0, 12.19)
Inhalation flow ($Lmin^{-1}$)	**Mean difference (95% confidence interval) Following two inhalations**			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30 vs. 60	-11.38***(-16.12, -6.64)	-19.23***(-28.82, -9.64)	14.61*(-25.21, -4.00)	-23.54***(-33.78, -13.29)

Mean difference is significant *P<0.05 **P<0.01***P<0.001

Table 6.7 Statistical comparisons of differences in emitted dose (% nominal dose) from the four DPIs between one and two inhalations

Inhalation flow ($Lmin^{-1}$)	Mean difference (95% confidence interval) one versus two inhalations			
	Accuhaler	Easyhaler	Clickhaler	Turbuhaler
30	-10.10**(-6.68,-3.51)	-6.25**(-10.22, -2.27)	-9.70*(-17.75,-.64)	-6.41* (-10.10,-.72)
60	-8.92**(-14.82,-3.02)	-9.01***(-13.54, -4.48)	-7.93(-16.02; .15)	-10.08***(-14.44;-.72)

Mean difference is significant *P<0.05 **P<0.01***P<0.001

6.4 Discussion

Several in-vitro studies have shown that the inhalation flow has a significant influence on the dosage emission from dry powder inhalers (DPIs). For instance, in-vitro studies using the salbutamol Accuhaler®, Easyhaler® and Turbuhaler® (Palander et al, 2000), terbutaline Turbuhaler® (Malton et al, 1996; Ross and Schulzt, 1996) have highlighted flow dependent dose emission from these inhalers. Other in-vitro studies by Al-fadhl et at al., (2005) using the tiotropium Handihaler® (a single dose-capsule) and De Boer et al (1997) using the drug Spinhaler® (Spincap) have highlighted that dose emission is influenced by inhalation flow and that two inhalations are required for each dose to empty the capsule. Similarly the results of the in-vitro study presented in chapter 4 demonstrated flow dependent dose emissions from the Accuhaler®, Easyhaler®, Clickhaler® and Turbuhaler and that the total emitted dose from the inhalers is overall significantly greater ($p \leq 0.05$) with two inhalations than one inhalation per metered dose. Simulated flows at constant rates from a vacuum pump were used in these in-vitro studies which do not accurately represent an individual's inhalation profile through each DPI. Patients inhale at varying flows through DPIs and their acceleration rates will differ. The inspiratory effort by the patient generates a pressure drop across the inhaler, which is proportional to the inhalation flow because the resistance of the inhaler is a constant variable (Clark and Hollingworth, 1993).

An inhalation profile recorder (IPR) has been developed to capture profiles of pressure drop across the mouthpiece of the inhaler versus time when an individual inhales through the inhaler. The pressure profile can be converted into an inhalation flow profile when the resistance of the device is known (Clark and Hollingworth, 1993). Also individual inhalation rates through the currently marketed dry powder inhalers (DPIs) can be identified using the In-Check Dial. The results of a study by Tarsin et al (2000) has highlighted that the peak inspiratory flows through the Accuhaler and the Turbuhaler

measured electronically (using an IPR) is as accurate as the peak inspiratory flows measured by the In-Check Dial.

Furthermore, the reported ex-vivo studies on the dose emission characteristics from the Accuhaler® and the Turbuhaler® using an IPR that recorded inhalation profiles from asthmatic children (Bisgard et al. 1998), COPD patients (Burnell et al. 2001) and asthmatic patients (Tarsin et al, 2006) with the Electronic Lung Device connected to a cascade impactor have highlighted flow dependent dose emission.

The data obtained from the current ex-vivo dose emission study are in Tables 6.1 to 6.4. These and Figures 6.2 to 6.5 show that the total emitted dose (% nominal dose) from the Accuhaler, Easyhaler, Clickhaler and Turbuhaler increased with respect to the inhalation flow. The data in Table 6.5 show the mean (SD) total emitted dose (% nominal dose) of the four inhalers following one and two inhalations. Statistically the total emitted dose from the inhalers significantly ($p \leq 0.001$) increased with the inhalation flow (Table 6.6). The results of this ex-vivo dose emission study demonstrate flow dependent dose emission similar to the results of the ex-vivo electronic lung studies cited above.

Furthermore, Figure 6.6 shows that the mean (SD) total emitted dose (% nominal dose) from the Accuhaler®, Easyhaler®, Clickhaler® and Turbuhaler® was generally greater when using two inhalations than one inhalation per metered dose at each inhalation flow. Statistical comparison confirmed significant ($p \leq 0.001$) differences in the total emitted dose from the inhalers between one and two inhalations per metered dose (Table 6.7) after one and two inhalations per dose. The results are similar with the results obtained in the in-vitro study presented in chapter 4. The ex-vivo methodology in this study cannot be extended to measure fine particle dose from the four studied inhalers.

6.5 Conclusion

Measurement of dose emission from dry powder inhalers by the in-vitro method at fixed flows does not accurately represent how individuals inhale through a device. The results

obtained using this method demonstrated the flow dependent dose emission of the four studied inhalers. The data obtained is consistent with the emitted dose from the Accuhaler and the Turbuhaler when the ex-vivo method using the Electronic Lung Device with recorded inhalations profiles from patients (Burnell et al, (2001); Tarsin et al., 2001). Thus, the total emitted dose obtained using this novel ex-vivo approach represented the emitted dose that an individual volunteer would have received when they inhaled through the Accuhaler®, Easyhaler®, Clickhaler® and Turbuhale®r.

Furthermore, the results obtained using this ex-vivo study highlights that the total emitted dose from the inhalers is significantly ($p \leq 0.001$) greater with two inhalations than one inhalation per metered dose, consistent with the results presented in chapter 4.

Chapter 7

7 General discussion and conclusion

7.1 General discussion

The inhaled route of administration is a preferred route for local treatment of lung diseases, including asthma and chronic obstructive pulmonary disease. The success of inhaled therapy depends on the ability of an inhaler to emit an adequate aerosolised dose with particles in an optimal size range for deposition to the appropriate sites in the lung (Pauwels et al, 1998). This, in turn, depends on the patient's inspiratory flow, inhaled volume and degree of airway obstruction (Martin et al. 1988; Stocks 1995). Thus, lung deposition from an inhaler is influenced by the inhalation technique used. Inhalers have different formulation designs that provide varying degrees of resistance to inhalation flow and require different inhalation techniques. All currently available dry powder inhalers (DPIs) are passive devices which rely on turbulent energy, generated by the interaction between the patient's inspiratory flow and resistance within the device, to emit drug fine particles that have the potential lung deposition. The patient's inspiratory flow is proportional to the turbulent energy, the resistance within the device being a constant variable (Clark and Hollingsworth, 1993). Thus, DPIs will deliver a larger lung dose at a high inhalation flow than at a low flow (Newman et al. 1991; Borsgstrom 1994; Pitcairn et al. 1994). Hence patient information leaflets for DPIs recommend the inhalation manoeuvre should be fast from the start of the inhalation, and sustained for as long as possible. Studies have highlighted that some patients have problems achieving a fast rate during routine use with a DPI. These studies have revealed that young children and those with severe obstruction are most likely to have problems using a fast inhalation flow (Peddersen et al. 1990; Broeder et al. 2003). Generally, during routine use patients will inhale through a variety of DPIs at varying inhalation flows and inhalation volumes.

This research programme has focused mainly on the use of the ex-vivo and in-vitro methods to identify the effect and influence of the inhalation flow, inhalation volume and

the number of inhalations per metered dose on the dose emission and aerodynamic dose emission characteristics of four different multidose dry powder inhalers (DPIs). The DPIs used were the salbutamol Accuhaler®, Easyhaler® and Clickhaler® as well as the terbutaline Turbuhaler®.

The performance of an inhaled product is assessed in terms of its total emitted dose and the aerodynamic characteristics. These parameters can be quantified using high performance liquid chromatography (HPLC) methods. Thus, a modified HPLC method for the assay of salbutamol sulphate and terbutaline sulphate in aqueous samples has been validated according to the ICH guidelines (ICH 1994) (chapter 3). The results of the validation highlighted that the assays were precise, accurate and sensitive to determine the amounts of salbutamol sulphate and terbutaline sulphate in aqueous samples from the dose emitted from the inhalers.

The total emitted dose from the Accuhaler®, Turbuhaler®, Clickhaler® and Easyhaler® has been evaluated with respect to inhalation flow, inhalation volume and the number of inhalations per metered dose (chapter 4).

Several in-vitro studies have shown that the emitted dose from different DPIs varies with a change in the inhalation flow to a varying extent (de Boer et al. 1996; Malton et al. 1995; Palander et al, 2000; Tarsin et al, 2004).The results identified in chapter (4) indicated that the total emitted dose (% nominal dose) from the Accuhaler®, Easyhaler®, Clickhaler® and Turbuhaler® increased with respect to the inhalation flow when tested using the same inhalation volume with one or two inhalations for each dose as shown in Figures 4.2 to 4.5. The extent of the variation was different for each type of device studied. The data in Tables 4.10 and 4.11 shows that although the emitted dose increased upon increasing the inhalation flow, generally there was no statistically significant difference with the Accuhaler® and the Easyhaler® over the inhalation flow range of 30 - 60 $Lmin^{-1}$. This is applied when inhaled volumes of 2L and 4L were used with the Accuhaler® and the

Easyhaler®. However, the flow dependent dose emission property of the Accuhaler® and the Easyhaler® was significant ($p<0.05$) at the inhalation flow range from 10 to 30 $Lmin^{-1}$ for 2L and 4L inhaled volumes. The Clickhaler® and the Turbuhaler® showed significant ($p<0.001$) flow dependent dose emission property throughout the inhalation flow range of 10 to 60$Lmin^{-1}$ when tested using 2L and 4L inhaled volumes. De Boer et al (1996) have attributed these differences in behaviour to differences in device-design and the type of powder formulation. The results of this study are consistent with the previous in-vitro study by Palander et al (2000) which highlights that the total emitted dose from the Accuhaler® and Easyhaler® are less affected by changes in inhalation flows than the Turbuhaler®.

The data in Table 4.12 shows overall, there were marginal differences in the emitted dose between 2L and 4L inhalation volumes at each inhalation flow for the four studied inhalers. The Accuhaler contains multiple doses with each dose factory dispensed dose in a blister on a strip inside the device, while the Turbuhaler®, Clickhaler® and Easyhaler® are the reservoir-type of multidose inhalers. This finding is consistent with a previous study by De Boer et al (1996) who found that the effect of inspiration time (inhalation volume) on the dose emission from the Turbuhaler® (reservoir) and the Diskhaler® (blister) over the same flow range was negligible for all four inspiration times (0.5, 1.5, 3.0 and 6 s) used. Although it was expected that a higher emitted dose may be obtained with a larger inhaled volume (because of the greater energy input), no significant difference was observed in this study. This may be attributed to particles being lifted from dose metering cup/strip and carried ex-mouthpiece of an inhaler early in the simulated inhalation. Previously Everard et al (1997) have reported that dose emission from DPIs formulated with either a reservoir or blister occurs immediately at the start of the inhalation. Thus, for these types of DPIs energy impacted on the powder to overcome attractive forces between particles, or the particle and carrier, by a 2L volume may not be significantly different from that provided

by a 4L inhaled volume. Hence, the effect of inhalation volume on the emitted dose is negligible highlighting the acceleration effect reported by Everard et al (1997).

The data describing statistical comparisons are in Tables 4.13 and 4.14. The emitted dose from the Accuhaler® was significantly ($p<0.05$) greater when using two inhalations than one inhalation for each dose at inhalation flows below 30 $Lmin^{-1}$. However, this trend was variable with the Turbuhaler. At the flow range of 30 to 60 $Lmin^{-1}$ using a 4L inhaled volume, the emitted dose was significantly ($p<0.05$) greater with two inhalations than one inhalation while for a 2L inhaled volume this trend occurred at the flow range of 10 to 30 $Lmin^{-1}$. On the other hand, the emitted dose from the Easyhaler and the Clickhaler was significantly ($p<0.001$) greater with two inhalations than one inhalation across the inhalation flow range of 10 to 60 $Lmin^{-1.}$

The aerodynamic dose emission characteristics of the four inhalers, especially the fine particle dose (FPD) that determines the lung deposition has been similarly evaluated (Chapter 5). The variability of the FPD (% nominal dose) with the inhalation flow following one inhalation for 2L and 4L inhaled volumes is shown in Figures 5.18 to 5.21. Similar data were respectively obtained for the inhalers following two inhalations (Table 5.18). Two inhalations per dose are therefore not required. Statistical comparison of the differences in the FPD between inhalation flows presented in Tables 5.19 and 5.20 has confirmed that the FPD significantly ($p<0.001$) increased with the increase in the inhalation flow. Overall, the results of this study have demonstrated the flow dependent dose emission property of the four inhalers, with the salbutamol Accuhaler® and Easyhaler® less affected than the salbutamol Clickhaler® and the terbutaline Turbuhaler®. Also the finding is in agreement with the previous reported in-vitro studies on the flow dependent FPD characteristics of salbutamol Accuhaler® and Easyhaler® (Palander et al. 2000) as well as the terbutaline Turbuhaler® (Malton et al. 1996; Ross and Schulz, 1996).

The mass median aerodynamic diameter (MMAD) provides an indication of the size distribution of drug particles deposited in the lower stages of the impactor (and hence the likely drug deposition site in vivo). The results show that the MMAD decreased with an increase in the inhalation flow. This is due to de-aggregation of the drug-carrier complex or in the case of the Turbuhaler® the pure pellets into fine particles thereby enhancing penetration to the lower stages of the impactor (Figures 5.22 to 5.25).

For each DPI, there will be a minimum inhalation flow (hence threshold energy) for dose emission containing particles with sufficient potential for lung deposition when patients inhale fast as they can. The data in Tables (5.2 to 5.17) show that the minimum inhalation flow for the Accuhaler®, Easyhaler® and Clickhaler® is 20Lmin^{-1}, while that for the Turbuhaler is about 30Lmin^{-1}. However, this in-vitro data should be viewed with caution as the data obtained from cascade impaction does not always reflect the clinical situation, nevertheless the data can be used to guide clinical response (Barry and O`Callaghan 2003).

The inhalation flow through a DPI is affected by its intrinsic resistance. Studies have shown (Tarsin, et al, 2000) that the Accuhaler has low resistance, whereas the Clickhaler®, Turbuhaler® and the Easyhaler® all have high resistance. Patients with COPD have been reported to have lower inhalation flows than adult asthmatics. Also the more severe the obstruction of the airways the lower were the inhalation flows through a variety of inhalers (Tarsin et al, 2001). Thus, the efficacy of an inhaled product will be affected by the age and abilities of the patient.

Some asthmatic children and COPD patients were unable to generate the minimum inhalation flow of 30 Lmin^{-1} through a Turbuhaler® (Pedersen et al. 1990; Broeder et al. 2003). The finding about the terbutaline Turbuhaler in this study implies that some patients with asthma//COPD may not be able generate a dose containing many particles with the potential for lung deposition from the Turbuhaler to achieve control. Thus, once their inhalation technique is identified with the aid of the In-Check Dial®, they should be

given intensive training if they still prefer the Turbuhaler or an alternative inhaler that suits their natural technique should be prescribed.

The Accuhaler® has low resistance to flow and thus less effort is required to achieve any given inspiratory flow. Clinical study (Conway et al. 1996) with asthmatic children and adults using the Accuhaler® has demonstrated that the minimum inhalation flow identified for the device is easily achievable. Hence, the force of inhalation may not be a critical factor when training patients to correctly use the Accuhaler. Although the Easyhaler® has a high resistance, a clinical study with asthmatic children using the device demonstrated that its clinical efficacy is not affected by low inspiratory flows (even as low as 16 $Lmin^{-1}$) (Koskela et al. 2000). Similar clinical studies with asthmatic children using a Clickhaler (intermediate resistance inhaler) demonstrated that its clinical efficacy is independent of inspiratory flows in the range of 15 to 60 $Lmin^{-1}$ (Newhouse et al. 1999). Thus, the Easyhaler® and the Clickhaler® can be used with confidence in patients who may have difficulty in generating a high inspiratory flow such as children and the elderly.

The FPD (% nominal dose) emitted from the Accuhaler®, Easyhaler®, Clickhaler® and the Turbuhaler® are generally similar at each inhalation flow for 2L and 4L except for slight differences (Figure 5.26). However, these differences were not statistically significant in most cases as shown in Table 5.22, highlighting the effect of acceleration rate reported by Everard et al, (1997.

Figures 5.27 and 5.28 have compared the fine particle dose (FPD) from the Clickhaler® and the Turbuhaler® at different inhalation flows with one and two inhalations per metered dose. There is no statistically significant difference ($p<0.05$) in most cases (Table 5.22). Overall, the influence of the number of inhalations on the fine particle dose hence lung deposition and ultimately clinical effects is negligible.

The results of the present ex-vivo study using inhalation flow from adult volunteers with the use of the In-Check Dial® (Chapter 6) indicated that the total emitted dose increased

with the inhalation flow (Figures 6.2 to 6.3). Statistically the total emitted dose from the inhalers significantly ($p \leq 0.01$) increased with the inhalation flow (Table 6.6) thereby demonstrating flow dependent dose emission of the four studied inhalers. These results are consistent with other ex-vivo studies using an inhalation flow profile recorder with the Electronic Lung Device ® (ELD). Thus, the total emitted dose obtained using the novel ex-vivo approach represented the amount of drug an individual would have received when they inhaled through each of the four inhalers.

7.2 Conclusion

The total emitted dose and the fine particle dose (FPD) emitted from the Accuhaler®, Easyhaler®, Clickhaler®, and the Turbuhaler® were dependent on the inhalation manoeuvre to a varying extent. Also the minimum inhalation flow has been identified to generate a fine particle dose with the greatest potential for lung deposition when patients inhale as fast as they can for the four studied inhalers. These values together with the use of the In-Check Dial® provide insights into the choice of an inhaler that suits a patient's natural technique for effective lung deposition.

Furthermore, the total emitted dose and fine particle dose from the four DPIs were overall not affected by an inhalation volume above 2L irrespective of the inhalation flow used. Since asthmatic patients have an average inhalation volume of about 2L when they inhaled through a DPI and they are the ultimate users of these inhalers, 2L rather than 4L recommended by the compendial methods should be considered for the in-vitro measurement of the total emitted dose and fine particle dose from these types of inhalers.

Although the four studied inhalers generally showed a significantly greater total emitted dose with two inhalations than one inhalation per metered dose as reported in chapter 4, overall there were no statistically significant differences in their fine particle dose between one and two inhalations per metered dose. Since it is the fine particle dose that is an indicator of lung deposition and ultimately the clinical effects, the finding in this study

tends to support the continuation of the present manufacturers` instructions for patients to inhale once for each metered dose `as fast as they can`.

A novel ex-vivo approach for the determination of dose emission of the four different DPIs, using the adult volunteer inhalation flows measured by the use of the In-Check Dial®, has been identified. The results of the ex-vivo study using the four different inhalers have demonstrated flow dependent dose emission. Thus, the total emitted dose obtained using the novel ex-vivo approach represented the amount of drug an individual would have received when they inhaled through each of the four inhalers. The ability of the volunteer to achieve high and low inhalation flow highlights the usefulness of the In-Check Dial® as a trusting aid.

7.3 Future work

The Thesis has shown that salbutamol delivered from an Accuhaler®, Clickhaler® and Easyhaler® as well as terbutaline from a Turbuhaler® all demonstrate flow dependent dose emission. These in-vitro results have shown that there is no difference between an inhalation volume of 2 and 4 L and that there was no need to make two inhalations from each dose. There is a greater difference between one and two inhalations in the results of the ex-vivo study. The latter use humans to generate the inhalation profile through an inhaler, whereas the in-vitro methods use a vacuum pump. When using the vacuum pump the acceleration of flow for a set peak inhalation flow will be the same but this will vary amongst humans. Recently work (at the University) has shown that formoterol formulated in a Turbuhaler® and an Easyhaler® does show a difference between 2 and 4 L inhalations. In the absence of this affect in this Thesis then drug : excipient ratio may be an issue. Also other recent clinical work is showing that many patients inhale with volumes down to 300ml with low inhalation flows.

Future work should be extended to:

1. Professor Chrystyn's research team is internationally recognised for their urinary pharmacokinetic methods to identify the relative lung and systemic bioavailability of salbutamol and terbutaline following an inhalation. The ex-vivo method could be repeated to include these samples. This was not studied in this thesis due to the lack of time. This study should be completed and will allow a comparison between the total dose emissions by in-vitro methods in chapter 4 to be compared to those using the ex-vivo method in Chapter 6 and to the in-vivo urinary pharmacokinetic methods. The ex-vivo and in-vivo studies should include inhalations using a slow and fast acceleration to attain the fast and slow peak inhalation flows that were used in the Thesis. All this work would expand the knowledge about in-vitro, ex-vivo and in-vivo correlations and provide some preliminary evidence about the influence of acceleration rates through DPI.
2. If the affect of inhalation flow and volume is dependent on the drug : excipient ratio then specially prepared formulations of salbutamol and terbutaline could be made. For example formulations of salbutamol 25, 50 and 100µg per dose and terbutaline 50 and 100µg per dose. This would be easily achieved using the Clickhaler® and the Easyhaler®. Special formulations in an Accuhaler and a Turbuhaler would have to be prepared by the manufacturer of these products. Nevertheless repeating the work in this thesis with the specially prepared low dose formulations in the Clickhaler® and the Turbuhaler® would provide the necessary evidence whether it is formulation that is a deciding factor for the effect of inhalation flow and volume.
3. Since many patients inhale with volumes below 2L then the same in-vitro studies as those in this thesis using flows from 20 -60 l/min with inhalation volumes of 500ml and 1L should be carried out. Also with formulations

prepared according to point 2 immediately above should be carried out with these smaller volumes.

4. Rather than use a vacuum pump the use of replaying inhalation profiles into the supplementary arm of the mixing inlet should be investigated. When the supplementary air into the mixing inlet is the same as that drawn through the ACI then replaying an inhalation profile into the supplementary air would result in the profile being drawn through the DPI. This would require a significant investment of equipment and setup as well as validation.

5. From the results in this Thesis it can be stated that the minimum flow through the Accuhaler®, Clickhaler® and Easyhaler® is 20 L/min and 30 L/min through a Turbuhaler®. The studies mentioned above would consolidate these claims. These could then be validated in clinical studies using patients inhaling at different flows and volumes. The focus would be low flows and small volumes.

8 References

Adams, N., Bestall, J. and Jones, P. W. (2001). Inhaled fluticasone proprionate forchronic asthma (Cochrane Review). In: The Cochrane Library 3: CD003135.

Akinbami, L. and Schoendorf, K. (2002). Trends in childhood asthma: Prevalence, healthcare utilization, and mortality. Paedriatrics 110: 315-322.

Al-Amoud, A. I., Clark, B. J. and Chrystyn, H. (2002). Determination of gentamicin in urine samples after inhalation by reversed-phase high-performance liquid chromatography using pre-column derivatisation with o-phthalaldehyde. J Chromatogr B Analyt Technol Biomed Life Sci 769(1): 89-95.

Al-Fadhl, SA, Assi, KH, Clark, B and Chrystyn, H. (2005). Tiotropium dose emission is influenced by the inhalation flow and recommended two inhalations. Eur Resp. J. 26 (Supl 49: 125S-126S.

Allen, T. (1990). Sampling and sizing from the atmosphere. Particle size measurement. New York, Chapman and Hall. 1: 78-80.

Altman, L. C., Hill, J. S., Hairfield, W. M. and Mullarkey, M. F. (1981). Effects of corticosteroids on eosinophil chemotaxis and adherence. J Clin Invest 67(1): 28-36.

Amirav, I., Newhouse, M. T. and Mansour, Y. (2005). Measurement of peak inspiratory flow with in-check dial device to simulate low-resistance(Diskus) and high-resistance(Turbohaler) dry powder inhalers in children with asthma. Pediatric Pulmonology 39(5): 447-451.

Analytical Profiles (1990). Terbutaline: Analytical profiles of drug substances. London, Academy of Pharmaceutical Sciences. Pharmaceutical Analysis and Control Section. Academic press, Inc. 19: 601-625.

Ashurst, I., Malton, A., Prime, D. and Sumby, B. (2000). Latest advances in the development of dry powder inhalers. PSTT 3(7): 246-256.

Assi, K .and Chrystyn, H. (2001). The different resistance of dry powder inhalers (DPIs). American Journal of Respiratory and Critical Care Medicines. 163: A443.

Aswania, O. and Chrystyn, H. (2001). Relative lung bioavailability of generic sodium cromoglycate inhalers used with and without a spacer device. Pulm Pharmacol Ther, 14(2), 129-33.

Aswania, O. and Chrystyn, H. (2002). Relative lung and systemic bioavailability of sodium cromoglycate inhaled products using urinary drug excretion post inhalation. Biopharm Drug Dispos 23(4): 159-63.

Aswania, O. A., Corlett, S. A. and Chrystyn, H. (1997). Development and validation of an ion-pair liquid chromatographic method for the quantitation of sodium cromoglycate in urine following inhalation. J Chromatogr B Biomed Sci Appl 690(1-2): 373-378.

Aswania, O. A., Corlett, S. A. and Chrystyn, H. (1998). Validation of a high-performance liquid chromatography assay for urinary nedocromil sodium following oral and inhaled administration. J Chromatogr B Biomed Sci Appl 718(2): 290-295.

Aswania, O. A., Corlett, S. A. and Chrystyn, H. (1999). Relative bioavailability of sodium cromoglycate to the lung following inhalation, using urinary excretion. Br J Clin Pharmacol 47(6): 613-8.

Barnes, N. C., Qiu, Y.-S., Pavord, I. D., Parker, D., Davis, P. A., Zhu, J., Johnson, M., Thomson, N. C., Jeffery, P. K. and on behalf of the, S. C. O. S. G. (2006). Antiinflammatory Effects of Salmeterol/Fluticasone Propionate in Chronic Obstructive Lung Disease. Am J Respir Crit Care Med 173(7): 736-743.

Barnes, P. J. (1995). Beta-adrenergic receptors and their regulations. Am.J. Respir.Crit. Care Med 152:838-860.

Barnes, P. J. (2000). Chronic obstructive pulmonary disease. N Engl J of Med 343(4): 269-280.

Barnes, P. J. and Pride, N. B. (1983). Dose-response curves to inhaled beta-adrenoceptor agonists in normal and asthmatic subjects. Br J Clin Pharmacol 15(6): 677-82.

Barrowcliffe, J., McGlynn, P., Ratcliffe, S., Sewell, M., Sheik, S. and Walters, M. (1997). Beclomethasone dipropionate (BDP) Clickhaler®. In-vitro performance of a steroid novel dry powder inhaler. In: The Society (eds). Drug Delivery to lungs V111. The Aerosol Society, Bristol. December 1997 125-128.

Barry, P. W. and O'Callaghan, C. (2003). The influence of inhaler selection on efficacy of asthma therapies. Adv Drug Deliv Rev 55(7): 879-923.

Becker, A. B. and Simons, F. E. (1989). Formoterol, a new long-acting selective beta 2-adrenergic receptor agonist: double-blind comparison with salbutamol and placebo in children with asthma. J Allergy Clin Immunol 84: 891-895.

Berridge, M., Lee, Z., Leisure, G., Moraldi, F. and Heald, D. (1998). Nasal delivery and kinetics of fluticasone propionate using positron tomography (PET). Pharm Sci 1(Suppl 4): S208-S209.

Bisgaard, H., Klug, B. and Sumby, B. S. (1998). Fine particle mass from Diskus inhaler and Turbuhaler inhaler in children with asthma. Eur. Resp. J 11:1111-1115.

Black, L. F. and Hyatt, R. E. (1969). Maximal respiratory pressures: Normal values and relationship to age and sex. Am Rev Respir Dis 99: 696-702.

Bondesson, E., Bengtsson, T., Borgstrom, L., Nilsson, L.E., Norrgren, K., Trofast, E. and Wollmer, P. (2003). Planar gamma scintigraphy-points to consider when quantifying pulmonary dry powder aerosol deposition. Int J Pharm 251(1-2): 33-47.

Borgstrom, L. and Nilsson, M. (1990). A method for determination of the absolute pulmonary bioavailability of inhaled drugs: terbutaline. Pharm Res 7(10): 1068-70.

Borgstrom, L., Newman, S., Weisz, A. and Moren, F. (1992). Pulmonary deposition of inhaled terbutaline: comparison of scanning gamma camera and urinary excretion methods. J Pharm Sci 81(8): 753-755.

Borgstrom, L. and Newman, S. (1993). Total and regional lung deposition of terbutaline sulphate inhaled via a pressurised MDI or via Turbuhaler(R). Int J Pharm 97(1-3): 47-53.

Borsgstrom, L. (1994). Deposition patterns with the Turbuhaler. J Aerosol Med 7 (suppl 1), S49-S53).

Boulet, L. P., Cowie, R., Johnston, P., Krakovsky, D. and Mark, S. (1995). Comparison of Diskus® inhaler, a new multidose powder inhaler, with Diskhaler®inhaler for the delivery of salmeterol to asthmatic patients . J of Asthma 32(6), 429-436.

Boyd, G. (1995). The continued need for MDIs. J Aerosol Med 8 (Suppl 1) : S 9-12.

British Pharmacopoeia (2005). Preparations for inhalation. Aerodynamic assessment of fine particles-fine particle dose and particle size distribution (Ph. Eur. method 2.9.18). British Pharmacopoeia. Stationery Office. London. 4: A277-290.

British Pharmacopoeia (2005). Terbutaline. British Pharmacopoeia. London, Stationery Office. 2: 1917-1918.

British Thoracic Society (2007). British guideline on the management of asthma. Thorax: 58 Suppl. I:1-98.

Britton, J., Hanley, S. P., Garrett, H. V., Hadfield, J. W. and Tattersfield, A. E. (1988). Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. Thorax 43(4): 300-305.

Brocklebank, D., Wright, J. and Cates, C. (2001). Systemic review of clinical effectiveness of pressurised metered dose inhalers versus other hand inhaler devices for delivering corticosteroids in asthma. BMJ (Clinical Research Ed) 323(7318): 896-900.

Broeders, M., Molema, J., Vermue, N. A. and Folgering, H. T. M. (2001). Peak inspiratory flow rate and slope of the inhalation profiles in dry powder inhalers, Eur Respir J. 18: 780-783.

Broeders, M. E., Molema, J., Hop, W. C. and Folgering, H. T. (2003). Inhalation profiles in asthmatics and COPD patients: reproducibility and effect of instruction. J Aerosol Med 16(2): 131-41.

Bruschi, C., Cerveri, I., Zoia, M. C., Fanfulla, F., Fiorentini, M., Casali, L., Grassi, M. and Grassi, C. (1992). Reference values of maximal respiratory mouth pressures: a population-based study. Am Rev Respir Dis 146: 790-793

Burge, P. S., Calverley, P. M. A., Jones, P. W., Spencer, S., Anderson, J. A. and Maslen, T. K. (2000). Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. BMJ 320(7245): 1297.

Burnell, P., Small, T., Doig, S., Johal, B., Jenkins, R. and Gibso, G. (2001). Ex-vivo product performance of Diskus and Turbuhaler inhalers using inhalation profiles from patients with severe chronic obstructive pulmonary disease. Respiratory Medicine 95, 324-330.

Busse, W. W. and Lemanske, R. F. J. (2001). Asthma. N Eng J Med 344: 350-362.

Calverley, P., Pauwels, R. and Vestbo, J. (2003). Combining salmeterol and fluticasone in the treatment of chronic obstructive pulmonary disease. Lancet 361: 449–456.

Cartairs, J., Nimmo, A. and Barnes, P. (1989). Autoradiographic visualization of beta-adrenoreceptor subtype in human lung. Am. Rev. of Resp.Dis. 132: 541-547.

Casterline, C. L., Evans 3rd, R. and Ward Jr, G. W. (1976). The effect of atropine and albuterol aerosols on the human bronchial response to histamine. J Allergy Clin Immunol 58(5): 607-13.

Cazzola, M. and Dahl, R. (2004). Inhaled combination therapy with long-acting {beta}2-agonists and corticosteroids in stable COPD. Chest 126(1): 220-237.

Cazzola, M., Marco, F. D., Santus, P., Boveri, B., Verga, M., Matera, M. G. and Centanni, S. (2004). The pharmacodynamic effects of single inhaled doses of formoterol, tiotropium and their combination in patients with COPD. Pulm Pharmacol Ther 17(1): 35-39.

Cazzola, M., Noschese, P., Salzillo, A., De Giglio, C., D'Amato, G. and Gabriella Matera, M. (2005). Bronchodilator response to formoterol after regular tiotropium or to tiotropium after regular formoterol in COPD patients. Respir Med 99(5): 524-528.

Celga, U. H. U. H. (2004). Pressure and inspiratory flow characteristics of dry powder inhalers. Respir. Med 98(Suppl 1), S22-S28.

Celli, B. R., MacNee, W., Agusti, A., Anzueto, A., Berg, B., Buist, A. S., Calverley, P. M. A., Chavannes, N., Dillard, T., Fahy, B., Fein, A., Heffner, J., Lareau, S., Meek, P., Martinez, F., McNicholas, W., Muris, J., Austegard, E., Pauwels, R., Rennard, S., Rossi, A., Siafakas, N., Tiep, B., Vestbo, J., Wouters, E. and ZuWallack, R. (2004). Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. Eur Respir J 23(6): 932-946.

Chege, J. K. and Chrystyn, H. (2000). The relative bioavailability of salbutamol to the lung using urinary excretion following inhalation from a novel dry powder inhaler: the effect of inhalation rate and formulation. Respir Med 94(1): 51-6.

Chrystyn, H. (2000). Methods to determine lung distribution of inhaled drugs - could gamma scintigraphy be the gold standard? Br J Clin Pharmacol. 49(6): 525-528.

Chrystyn, H. (2001). Methods to identify drug deposition in the lungs following inhalation. Br J Clin Pharmacol. 51(4): 289-299.

Chrystyn, H. (2003). Is inhalation rate important for a dry powder inhaler? using the In-Check Dial to identify these rates. Respir Med 97(2): 181-187.

Chrystyn, H. (2006). The Diskus: A review of its position among dry powder inhaler devices. Int.J Clin Pract 15.11.

CHSR (1999). Centre for Health Services Research - The primary care management of asthma in adults - North of England Evidence Based Guideline Development Project. Newcastle upon Tyne. University of Newcastle upon Tyne. Report No. 97.

Clark, A. (1994). Effect of powder inhaler resistance upon inspiratory profiles in health and disease. Respiratory Drug Delivery IV 117-123.

Clark, A. R. and Bailey, R. (1996). Inspiratory flow profiles in disease and their effects on the delivery characteristics of dry powder inhalers. Respiratory Drug Delivery V USA:Phoenix, Arizona V: 221-230.

Clark, A. R. and Hollingworth, A. M. (1993). The relationship between powder inhaler resistance and peak inspiratory conditions in healthy volunteers--implications for in vitro testing. J Aerosol Med 6(2): 99-110.

Clarke, S. (1990). Respiratory defences. In Brewis, R.A.L, Gibson,J.C and Geddes,D.M.Eds. . Respiratory medicine. Balliere Tindall, Londo: 176-189.

Cockcroft, D. W., Killian, D. N., Mellon, J. J. and Hargreave, F. E. (1977). Protective effect of drugs on histamine-induced asthma. Thorax 32(4): 429-437.

Conway, J., Smith, S. and Schreiber, J. (1996). Comparison of peak pressure drops through powder inhalers drug inspiration at maximum flow rate. Am J Respir Crit Care Med 153: A59.

Crompton, G. K. (1982). Problems patients have using pressurised aerosol inhalers. Eur. J. Respir. Dis. 119: 101-104.

Crompton, G. K. (1990). The adult`s difficulties with inhalers. Lung, (Suppl.168):658-662.

Dahl, R., Lundback, B., Malo, J. L., Mazza, J. A., Nieminen, M. M., Saarelainen, P. and Barnacle, H. (1993). A dose-ranging study of fluticasone propionate in adult patients with moderate asthma. International Study Group. Chest 104(5): 1352-1358.

Davies, R. J. (1998). Respiratory diseases. In: Kumar, PJ, Clark, ML ed. Clinical medicine. London: W.B Saunders: 745-828.

de Boer, A. H., Bolhuis, G. K., Gjaltema, D. and Hagedoorn, P. (1997). Inhalation characteristics and their effects on in vitro drug delivery from dry powder inhalers: Part 3: the effect of flow increase rate (FIR) on the in vitro drug release from the Pulmicort 200 Turbuhaler. Int J Pharm 153(1): 67-77.

de Boer, A. H., Gjaltema, D. and Hagedoorn, P. (1996). Inhalation characteristics and their effects on in vitro drug delivery from dry powder inhalers Part 2: Effect of peak flow rate (PIFR) and inspiration time on the in vitro drug release from three different types of commercial dry powder inhalers. Int J Pharm 138(1): 45-56.

de Koning, J. P., van der Mark, T. W., Coenegracht, P. M. J., Tromp, T. F. J. and Frijlink, H. W. (2002). Effect of an external resistance to airflow on the inspiratory flow curve." Int J Pharm 234(1-2): 257-266.

DeBoeck, K., Alifier, M. and Warnier, G. (1999). Is correct use of a dry powder inhaler (Turbuhaler) age dependent. Journal of Allergy and Clinical Immunolgy 103: 763-767.

Dekhuizen, P. N. R., Folgering, H. T. M. and Herwaarden, C. L. A. (1991). Target-flow inspiratory muscle training during pulmonary rehabilitation in patients with COPD. Chest 99: 128-133.

Dhand, R. and Fink, J. B. (1999). Dry powder inhalers. Respir Care 44(8): 940-951.

Ducharme, F. M. and Hicks, G. C. (2002). Anti-leukotriene agents compared to inhaled corticosteroids in the management of recurrent and/or chronic asthma. Cochrane Database Syst Rev Abstract(3).

Eaton, T., Lewis, C., Young, P., Kennedy, Y., Garrett, J. E. and Kolbe, J. (2004). Long-term oxygen therapy improves health-related quality of life. Respir Med 98(4): 285-293.

Eiser, N. M., Phillips, C. and Wooler, P. A. (2001). Does the mode of inhalation affect the bronchodilator response in patients with severe COPD? Respir Med 95(6): 476-483.

Emeryk, A., Bartkowiak-Emeryk, M. and Kokot, I. (2000). Assessment of inspiratory flow rate (PIFR) is advisable before choosing a dry powder inhaler for treatment of asthmatic children. Eur Resp J 10

(Suppl 31): 540s.

Engel, T., Scharling, B., Skovsted, B. and Heing, J. (1992). Effects,side effects and plasma cocentrations of terbutaline in adult asthmatics when inhaling from a dry powder inhaler device and different inhalation flows and volumes. Br J Clin Pharmacol., 33:439-444.

European Pharmacopeia (2007). Preparations for inhalation: aerodynamic assessment of fine particles. European Pharmacopoeia. 6th ed.: 287-300.

Everard, M. L., Devadason, S. G. and Le Soue, P. N. (1997). Flow early in the inspiratory manoeuvre affects the aerosol particle size distribution from a Turbuhaler. Respir Med. 91(10): 624-628.

Fink, J. B. (2000). Metered dose inhalers, dry powder inhalers and transitions. Respir Care 45(6):623-635.

Fink, J. B. and Rubin, B. (2005). Problems with inhaler use: A call for improved clinician and patient education. Respiratory Care 50 (10): 1360-1375.

Fiz, J. A., Montserrat, J. M., Picado, C., Plaza, V. and Agusti-Vidal, A. (1989). How many manoeuvres should be done to measure maximal inspiratory mouth pressure in patients with chronic airflow obstruction? Thorax 44: 419-421.

Fletcher, C. and Peto, R. (1977). The natural history of chronic airflow obstruction. Br Med J 1(6077): 1645-8.

Florey, K. (1990). Academy of Pharmacutical Sciences. Pharmaceutcal Analysis and Control Section. Terbutaline, In: Analytical profiles of drug substances. Vol. 19 London; Academic press, INC., pp. 601-625 .

G.K.Crompton (1982). Problems patients have using pressurised aerosol inhalers.Eur. J. Respir. Dis. 119: 101-104.

Giraud, V. and Roache, N. (2002). Misuse of corticordsteroid metered-dose inhaler is associated with decreased asthma stability. Eur Respir J 19: 246-251.

Global Initiative for Chronic Obstructive Lung Disease (GOLD). (2007). Global Strategy for Diagnosis, Management, and Prevention of COPD. Retrieved March 2008, from http://www.goldcopd.com.

Goldstein, D., Tan, Y. and Soldin, S. (1987). Pharmacokinetics and absolute bioavailability of salbutamol in healthy adult volunteers. Eur J. Clin. Pharmacol. 32; 631-634.

Gorecka, D., Gorzelak, K., Sliwinski, P., Tobiasz, M. and Zielinski, J. (1997). Effect of long-term oxygen therapy on survival in patients with chronic obstructive pulmonary disease with moderate hypoxaemia.Thorax 52(8): 674-679.

Green, R. J. and Harris, N. D. (2000). Pathology and therapeutics for pharmacists. London: The Pharmaceutical Press.

Gunawardena, K., Clay, M. and Jenkins, M. (1995). The Diskus/Accuhaler multi-dose powder inhaler for the delivery of salmeterol in adult asthmatics. British Medical Journal (Clinical Research Ed) 6; 57-61 6; 57-61.

Hagan, R. (1994). High performance liquid chromatography for small scale studies of drug stability. Am. L. Hosp. Pharm Sup 1; 51(17):2162-75.

Hawksworth, G. M., James, L. and Chrystyn, H. (2000). Characterization of the inspiratory manoeuvre when asthmatics inhale through a Turbohaler pre- and post-counselling in a community pharmacy. Respir Med 94(5): 501-504.

Hickey, A. J. (1992). Pharmaceutical inhalation aerosol technology. New York, Marcel Dekker, Inc.

Hickey, A. J. (1996). Inhalation aerosols: Physical and biological basis for therapy. New York, Marcel Dekker, Inc.

Hill, J. and Thomason, N. (1998). The changing epidemiology of asthma. Scot. Med. J 43:67-69.

Hillery, A. M., Lloyd, A. W. and Swarbrick, J. (2001). Pulmonary drug delivery. Drug delivery and targeting for pharmacists and pharmaceutical scientists. London, Taylor & Francis: 269-300.

Hindle, M. and Chrystyn, H. (1992). Determination of the relative bioavailability of salbutamol to the lung following inhalation. Br J Clin Pharmacol. 34(4): 311-315.

Hinds, W. C. (1982). Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. Wiley-Interscience. New York, John Wiley & Sons 442.

Hogg, P. J. C. (2004). Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet 364(9435): 709-721.

Hughes, J. M. P. and Pride, N. B. (2000). Lung function test: physiologyical principles and clinical applications. W.B. Saundrs, London.

Hunninghake, G. W. and Crystal, R. G. (1983). Cigarette smoking and lung destruction: Accumulation of neutrophils in the lungs of cigarette smokers. Am Rev Respir Dis 128(5): 833-8.

Hyder, J. (1982). Particle transport onto human airway surfaces. Eur J Resrpir Dis 63(Suppl 114) 29-50.

ICH (1994). The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use guidelines. Eu Ph. Technical Guide 1999.

Jensen, E. J., Dahl, R. and Steffensen, F. (2000). Bronchial reactivity to cigarette smoke; relation to lung function, respiratory symptoms, serum-immunoglobulin E and blood eosinophil and leukocyte counts. Respir Med 94(2): 119-127.

K.Wetterlin (1998). Turbuhaler: A new powder inhaler for administration of drugs to the airways. Pharmaceutical Reasrch 5(8).

Kamiya, A., Sakagami, M., Hindle, M. and Byron, P. R. (2004). Aerodynamic sizing of metered dose inhalers: An evaluation of the Andersen and next generation pharmaceutical impactors and their USP methods. J Pharm Sci 93(7): 1828-1837.

Kay, A. B. (2001). Allergy and allergic diseases: First of two parts. N Eng J Med 344: 30-37.

Keatings, V. M. and Barnes, P. J. (1997). Granulocyte activation markers in induced sputum: comparison between chronic obstructive pulmonary disease, asthma, and normal subjects. Am J Respir Crit Care Med 155: 449-453

Keatings, V. M., Collins, P. D., Scott, D. M. and Barnes, P. J. (1996). Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. Am J Respir Crit Care Med 153: 530-534

Keller, M. (1999). Innovations and perspectives of metered dose inhalers in pulmonary delivery. Inter. J of Pharm 186(1), 81-90.

Kips, J. C. and Pauwels, R. A. (2001). Long-acting inhaled beta(2)-agonist therapy in asthma. Am J Respir Crit Care Med 164: 923-932.

Koskela, T., Malmstrom, K. and Sairanen, U. (2000). Efficacy of salbutamol via Easyhaler® unaffected by low inspiratory flow. Respir Med 94: 1229-33.

Koulouris, N., Mulvey, D. A., Laroche, C. M., Green, M. and Moxham, J. (1988). Comparison of two different mouthpieces for the measurement of Pimax and Pemax in normal and weak subjects. Eur Respir J 1: 863-867.

Kresch, M. J., Lima, D. M.Lu, H (1996). Developmental regulation of phospholipid secretion by fetal type II pneumocytes. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism 1299(1), 34-46.

Larson, J. L., Covey, M. K., Vitalo, C. A., Alex, C. G., Patel, M. and Kim, M. J. (1993). Maximal inspiratory pressure: Learning effect and test-retest reliability in patients with chronic obstructive pulmonary disease. Chest 104: 448-453.

Leach, C., Davidson, P., Hasseiquist, B. and Boudrean, R. (2005). Influence of particle size and patient dosing technique on lung deposition of HFA-beclomethasone from a metered dose inhaler. Journal of Aerosol Medicine 18 (4): 379-385.

Leech, J. A., Ghezzo, H., Stevens, D. and Becklake, M. R. (1983). Respiratory pressures and function in young adults. Am Rev Respir Dis 128(17-23).

Leith, D. E. and Bradley, M. (1976). Ventilatory muscle strength and endurance training. J Appl Physiol 41(4): 508-516.

Lippmann, M., Yeates, D. M. and Abert, R. E. (1980). Deposition retention and clearnce of inhaled particles. Br J. Ind. Med. 37:337-362.

Lipworth, B. J. (1996). Pharmacokinetics of inhaled drugs. Bri. J Clin. Pharmcol 36:445-450.

Malton, A.,Sumby, B.S., Smith, I.J. (1995). A comparison of in-vitro drug delivery from two multidose powder inhalation devices. Eur J Clin Res 7: 177-193.

Malton, A., Sumby, B.S., Dandiker Y. (1996). A comparison of in-vitro drug delivery from salbutamol Diskus and terbutaline Turbuhaler inhalers. J Pharm Med 6 : 35-48

Martin, L. E., Hobson, J., Page, J. and Harrison, C. (1971). Metabolic studies of salbutamol-3H: A new bronchodilator, in rat, rabbit, dog and man. Eur J Pharmacol. 14: 183-199.

Martin, T., Feldman, H., Fredberg, J., Castile, R., Mead, J. and Wohl, M. (1988). Relationship between maximal expiratory flows and lung volumes in growing humans. Appl Physiol 65:822-828.

Martindale (2002). Bronchodilators and anti-asthmata drugs. Martindale : the complete drug reference. C. S. Sweetman. London, Pharmaceutical Society of Great Britain. Pharmaceutical Press: 757-786.

Mayos, M., Giner, J., Casan, P. and Sanchis, J. (1991). Measurement of maximal static respiratory pressures at the mouth with different air leaks. Chest 100: 364-366.

Melani, A., Zanchetta, D., Barbato, N., Sestina, P., Cinti, C. and Canessa, P. (2004). Inhalation technique and variables associated with misuse of conventional metered dose

inhalers and newer dry powder inhalers in experienced adults. Ann Allergy Asthma Immunol 93 (5): 439-446.

Mitchell, J. P. and Nagel, M. W. (2003). Cascade impactors for the size characterization of aerosols from medical inhalers: Their uses and limitation. J Aerosol Med 16: 341-376.

Mobley, C. and Hochhaus, G. (2001). Methods used to assess pulmonary deposition and absorption of drugs. Drug Discov Today 6(7): 367-375.

Moore, A. and Stone, S. (2004). Original Paper Meeting the needs of patients with COPD: patients` preference for the Diskus inhaler compared with the Handihaler. International Journal of Clinical Practice 58(5): 444.

Morgan, D., Paul, J., Richard, B., Wilson-Evered, E. and Ziccone, S. (1986). Pharmacokinetics of intraveneous and oral salbutamol and its sulphate conjugate. Br J. Pharmacol 22: 587-593.

Nadarassan, D. K., Chrystyn, H., Clark, B. J. and Assi, K. H. (2007). Dose emission of formoterol fumarate from a Turbuhaler using in-vitro and in-vivo methods. Postgraduate Studies in Pharmaceutical Technology, UK, School of Pharmacy, University of Bradford.

National Institute for Health and Clinical Excellence (NICE). (2004). Chronic obstractive pulmonary disease: national clinical guideline for management of chronic obstructive pulmonary disease in adults in primary and secondary care. Available from http://www.nice.org.uk/guidance/.[cited on 12 September 208].

Nava, S., Ambrosino, N., Crotti, P., Fracchia, C. and C., R. (1993). Recruitment of some respiratory muscles during three maximal inspiratory manoeuvres. Thorax 48: 702-707.

Newell, S. Z., McKenzie, D. K. and Gandevia, S. C. (1989). Inspiratory and skeletal muscle strength and endurance and diaphragmatic activation in patients with chronic airflow limitation. Thorax 44: 903-912.

Newhouse, M. T., Nantel, N. P., Chambers, C. B., Pratt, B. and Parry-Billings, M. (1999). Clickhaler (a novel dry powder Inhaler) provides similar bronchodilation to pressurized metered-dose inhaler, even at low flow rates. Am Coll Chest Phys 4: 115 (952-956).

Newman, S. P., Pavia, D. and Clarke, S. W. (1981). Improving the bronchial deposition of pressurised aerosols. Chest 80: 909-911.

Newman, S. P., Pavia, D., Garland, N. and Clarke, S. W. (1982). Effects of various inhalation modes on the deposition of radioactive pressurized aerosols. Eur J Respir Dis Suppl 119(suppl): 57-65.

Newman, S. (1982). The correct use of inhalers. In: Clark, T (ed). Steroids in asthma. Auckland: Adis Press. pp.210-216.

Newman, S. P. and Clarke, S. W. (1983). Therapeutic aerosol 1-Physical and practical considerations. Thorax 38: 881-886.

Newman, S., Moren, F., Trofast, E., Talaee, N. and Clarke, S. (1991). Terbutaline sulphate Turbuhaler: effect of inhaled flow rate on deposition and efficacy. International Journal of Pharmaceutics 74(2-3): 209-213.

Newman, S. P. (1991). Aerosol physiology, deposition and metered dose inhalers. Allergy Proc 12(1): 41-45.

Newman, S. P., Talaee, N. and Clarke, S. W. (1991). Salbutamol aerosol delivery in man with the Rondo Spacer. Acta Ther 17: 49-50.

Newman, S. P. and Wilding, I. R. (1999). Imaging techniques for assessing drug delivery in man. Pharm Sci Technolo Today 2(5): 181-189.

NHLBI (1997). National Asthma Education and Prevention Programme, Expert Panel Report 2. Guidelines for the diagnosis and management of asthma. NIH Publication No. 97-4051. Bathesda, MD, US Department of Health and Human services.

Nials, A. T., Ball, D. I., Butchers, P. R., Coleman, R. A., Humbles, A. A., Johnson, M. and Vardey, C. J. (1994). Formoterol on airway smooth muscle and human lung mast cells: a comparison with salbutamol and salmeterol. Eur J Pharmacol 251(2-3): 127-35.

NICE (2004). National Institute for Health and Clinical Excellence (NICE): Chronic obstructive pulmonary disease: national clinical guideline for management of chronic obstructive pulmonary disease in adults in primary and secondary care. Available from http://www.nice.org.uk/guidance/.

Nielsen, K., Auk, I. L. and Bojsen, K. (1998). Clinical effect of Diskus dry powder inhaler at low and high inspiratory flow rates in asthmatic children. Eur. Respir. J 11:350-354.

Nsour, W., Alfred, A., Corrado, O. and Chrystyn, H. (2001). Measurement of peak inspiration rates with an In-Check meter to identify an elderly patient`s ability to use a Turbuhaler. Respir Med 95: 965-968.

O'Byrne, P., Barnes, P. J., Rodriguez-Gomez, G., Runnerstrom, E., Sandstrom, T. and Svenson, K. (2001). Low dose inhaled budesonide and formoterol in mild persistent asthma: the OPTIMA randomized trial. Am J Respir Crit Care Med 164: 1392-1397.

O'Kroy, J. A. and Coast, J. R. (1993). Effects of flow and resistive training on respiratory muscle endurance and strength. respiration 60: 279-283.

Palander, A., Mattila, T., Karhu, M. and Muttonen, E. (2000). In-vitro comparison of three salbutamol-containing multidose dry powder inhalers. Clin. Drug Invest. 20: 25-33.

Pauwels R, Newman S and L., B. (1997). Airway deposition and airway effects of antiasthma drugs delivered from metered-dose inhalers. Eur Respir J 10: 2127-2138.

Pauwels, R. A., Buist, A. S., Calverley, P. M. A., Jenkins, C. R. and Hurd, S. S. (2001). Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease . NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop Summary. Am J Respir Crit Care Med 163(5): 1256-1276.

Pauwels, R. A., Pedersen, S. and Busse, W. W. (2003). Early intervention with budesonide in mild persistent asthma: a randomised, double-blind trial. Lancet 361(9363): 1071-1076.

Pedersen, S. (1987). Inhaler use in children with asthma. Dan Med Bull 34: 234-249.

Pedersen, S., Frost, L. and Arnfred, T. (1986). Errors in inhalation techniques and efficiency in inhaler use in asthmatic children. Allergy 41: 118-124.

Pedersen, S. and Steffensen, G. (1986). Fenoterol powder inhaler technique in children: influence of respiratory flow rate and breath-holding. Eur J Respir Dis 68: 207-214.

Petty, T. L. (1995). Enjoying life with chronic obstructive pulmonary disease. NJ, Cedar Grove Laennec Publishing.

Pitcairn, G., Lunghetti, G., Venturap, P. and Newman, S. (1994). A comparison of the lung deposition of salbutamol inhaled from a new dry powder inhaler, at two inhaled flow rates. Int J Pharm 102(1-3): 11-18.

Pitcairn, G., Lim, J., Hollingworth, A. and Newman, S.P (1997). Scintigraphic assessment of drug delivery for the Ultrahaler dry powder inhaler. J. Aerosol Med.;10: 295-306.

Poole, P. J. and Black, P. N. (2001). Oral mucolytic drugs for exacerbations of chronic obstructive pulmonary disease: systematic review. BMJ 322(7297): 1271.

Rainer, T., Saunders, M. and Richards, R. (1992). Determinants of inspiratory flow rate (IFR) through Diskhalers. Thorax 47: 239.

Raphael, G. D., Lanier, R. Q., Baker, J., Edwards, L., Rickard, K. and Lincourt, W. R. (1999). A comparison of multiple doses of fluticasone propionate and beclomethasone dipropionate in subjects with persistent asthma. J Allergy Clin Immunol 103(5 Pt 1): 796-803.

Rau, J. (2005). The inhalation of drugs: Advantages and problems. Respir Care. 50: 367-382.

Raul, J. (2006). Practical problems with aerosol therapy in COPD. Respir Care 51: 158-172.

Rees, J. E., Clark, T. J. H. and Moren, F. (1982). The importance of particle size response to inhaled bronchodilators. Eu, J,Respir. Dis.; 63 (Suppl 119); 73-78.

Reid, W. D. and Dechman, G. (1995). Considerations when testing and training the respiratory muscles. Phys Ther 75(11): 971-982.

Reid, W. D. and Samrai, B. (1995). Respiratory muscle training for patients with chronic obstructive pulmonary disease. Phys Ther 75(11): 996-1005.

Repine, J. E., Bast, A. and Lankhorst, I. (1997). Oxidative stress in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 156: 341-357

Retamales, I., Elliott, W. M., Meshi, B., Coxson, H. O., Pare, P. D., Sciurba, F. C., Rogers, R. M., Hayashi, S. and Hogg, J. C. (2001). Amplification of inflammation in emphysema and its association with latent adenoviral infection. Am J Respir Crit Care Med 164 (3): 469-473.

Rodriguez-Carballeira, M., Heredia, J. L., Rue, M., Quintana, S. and Gomez, L. (2001). The bronchodilator test with increasing doses of terbutaline in chronic obstructive pulmonary disease patients. Pulmonary Pharmacology & Therapeutics 14(1): 61-65.

Rönmark, E., Jögi, R., Lindqvist, A., Haugen, T., Meren, M., Loit, H., Sairanen, U., Sandahl, A. and Lundbäck, B. (2005). Correct use of three powder inhalers: comparison between Diskus, Turbuhaler, and Easyhaler. J Asthma. 42: 173-178.

Ross, D. L. and Schultz, R. K. (1996). Effect of inhalation flow rate on the dosing characteristics of dry powder inhaler (DPI) and metered dose inhaler (MDI) products. J Aerosol Med 9 (2): 215-26.

Sarinas, P. S. A., Robinson, T. E., Clark, A. R., Canfield, J., Chitkara, R. K. and Fick, R. B. (1998). Inspiratory flow rate and dynamic lung function in cystic fibrosis and chronic obstructive lung diseases. Chest 114(4): 988-992.

Savage, L. and Goodyer, L. (2003). Providing information on metered dose inhaler technique: is multimedia as effective as print. Family practice 20 (5): 552-557.

Sears, M. and Lotvall, J. (2005). Past, Present, and Future--[bete]2-adrenoceptor agonists in asthma management. Respiratory medicine. 99(2), 215-26.

Selroos, O., Pietenalho, A. and Riska, H. (1996). Delivery device for inhaled asthma medication. Clinical implications of differences in effectiveness. Clin. Immunother 6: 273-299.

Seppälä, O. P., Herrala, J., Hedman, J., Alanko, K., Liipo, K., Terho, E., Pietinalho, A., Nyholm, J. E. and Nieminen, M. M. (1998). The bronchoprotective efficacy of salbutamol inhaled from a new metered-dose powder inhaler compared with a conventional pressurized metered-dose inhaler connected to a spacer. Respir Med 92(3): 578-583.

Shenfield, G., Evans, M. and Paterson, J. (1976). Absorption of drugs by the lungs. Br. J. Clin. Pharmacol. 3:583-589.

SIGN (2002). Evidence table 4.4c: inhaled corticosteroid vs leukotriene receptor antagonists. Pharmacological management of asthma.

SIGN (2002). Pharmacological management of asthma, Evidence table 4.1: inhaled short acting beta 2 agonists. Edinburgh: Available from url: http://www.sign.ac.uk/guidelines/published/support/guideline63/index.html.

SIGN (2002). Pharmacological management of asthma. Evidence table 4.11b: add-on drugs for inhaled steroids: Long acting or oral β2 agonists. SIGN: Available from url: http://www.sign.ac.uk/guidelines/published/support/guideline63/index.html

Silkstone, V. (1999). In vitro and In-vivo studies to assess the performance of nebulisers. PhD Thesis. University Of Bradford.

Snell, J. and Ganderton, D. (1999). Assessing lung deposition of inhaled medications: Consensus statement from a workshop of the British Association of Lung Research. Respir Med 93: 123-133.

Srichana, T., Martin, G. P. and Marriott, C. (1998). Dry powder inhalers: The influence of device resistance and powder formulation on drug and lactose deposition in vitro. Eur J Pharm Sci 7(1): 73-80.

Stanescu, D., Sanna, A., Veritier, C., Kostianev, S., Calcagni, P. G., Fabbri, L. M. and Maestrelli, P. (1996). Airways obstruction, chronic expectoration and rapid decline of FEV_1 in smokers are associated with increased levels of sputum neutrophils. Thorax 51: 267-271

Steckel, H. and Muller, B. (1997). In vitro evaluation of dry powder inhalers 1: drug deposition of commonly used devices. Int J Pharm 154: 19-29.

Stocks, J. (1995). Developmental physiology and methodology. Am J Respir Crit Care Med 151:S15-S17.

Sturevany, J. (1999). NSAID induced bronchospasm- a common and serious problem, a report from MEDSAFE, The New Zealand Medicines and Medical devices Safety Authority. N Z Dent J 95: 84.

Svartengren, M., Andersen, M., Bylin, G., Philison, K. and Camner, P. (1991). Mouth and throat deposition of 3.6 μm radiolabelled particles in asthmatics. J Aerosol Med 4:313-321.

Sweetman, S. and Britain, P. S. O. G. (2002). Bronchodilators and Anti-asthma drugs. In: Martindale: the complete drug reference. London:Pharmaceutical Press, pp. 757-786.

Tarsin, W., Assi, K. and Chrystyn, H. (2004). In-vitro intra-and inter-inhaler flow rate dependent dosage emission from a combination of budesonide and eformoterol in a dry powder inhaler. J Aerosol Med 17:25-32.

Tarsin, W., Pearson, S., Assi, K. and Chrystyn, H. (2006). Emitted dose estimates from Seritide Diskus and Symbicort Turbuhaler following inhalation by severe asthmatics. Inter J. Pharm 316(1-2): 131-137.

Tarsin, W. Y. (2000). In-vivo, in-vitro and ex-vivo investigations to identify the influence of inhalation rate when patients use a dry powder inhaler. PhD Thesis. University Of Bradford.

Tarsin, W. Y., Assi, K., Corrado, O. and Chrystyn, H. (2000). Measurement of patient inhalation rates for different dry powder inhalers (DPIs) using the In-Check Dial. Thorax 55 (Suppl, 3): A63.

Tattersfield, A. E. (1987). Effect of beta-agonists and anticholinergic drugs on bronchial reactivity. Am Rev Respir Dis 136(4 Pt 2): S64-8.

Taylor, K. M. G. and McCallion, O. N. M. (1997). Ultrasonic nebulisers for pulmonary drug delivery. Int. J. of Pharmaceutics 153(1): 93-104.

Terzano, C. (2001). Pressurised Metered Dose Inhalers and Add-on Devices. Pulmonary Pharmacology and Therapeutics 14(5):351-366.

Tetley, T. D. (1993). New perspectives on basic mechanisms in lung disease. 6. Proteinase imbalance: its role in lung disease. Thorax 48: 560-565

Theophilus, A., Moore, A., Prime, D., Rossomanno, S., Whitcher, B. and Chrystyn, H. (2006). Co-deposition of salmeterol and fluticasone propionate by a combination inhaler. Int J Pharm 313(1-2): 14-22.

Timsina, M., Martina, G., Marriott, C., Ganderton, D. and Yianneskis, M. (1994). Drug delivery to respiratory tract using dry powder inhalers. Inter J Pharm 101: 1-13.

Tomlinson, H. S., Corlett, S. A., Allen, M. B. and Chrystyn, H. (2005). Assessment of different methods of inhalation from salbutamol metered dose inhalers by urinary drug excretion and methacholine challenge. Br J Clin Pharmacol 60(6): 605-610.

Tzelepis, G. E., Kadas, V. and McCool, F. D. (1999). Inspiratory muscle adaptations following pressure or flow training in humans. Eur J Appl Physiol 79: 467-471.

United States Pharmacopeia (2002). Terbutaline. The United States pharmacopeia 25 [and] The national formulary 20. The board of trustees: 1658-1660.

United States Pharmacopeia (2009). Aerosols, nasal sprays, metered dose inhalers and dry powder inhalers. The United States pharmacopeia 32 [and] The national formulary 27. The board of trustees: 204-224.

Van Beerendonk, I., Mesters, I., Mudde, A. and Tan, T. (1998). Assessment of the inhalation technique in outpatients with asthma or chronic obstructive pulmonary disease using a metered dose inhaler or dry powder device. Journal Asthma 35 (3): 273-279.

van Noord, J. A., Aumann, J. L., Janssens, E., Smeets, J. J., Verhaert, J., Disse, B., Mueller, A. and Cornelissen, P. J. G. (2005). Comparison of tiotropium once daily, formoterol twice daily and both combined once daily in patients with COPD. Eur Respir J 26(2): 214-222.

Van Oort, M. (1995). In vitro testing of dry powder inhalers. Aerosol Sci Technol 22(4): 364-373.

Vaswani.S.K. and Creticos, P. S. (1988). Metered dose inhaler: past, present, and future. Ann Allergy Asthma Immunol 80(1), 11-9; quiz 19-70.

Vestbo, J., Sorensen, T., Lange, P., Brix, A., Torre, P. and Viskum, K. (1999). Long-term effect of inhaled budesonide in mild and moderate chronic obstructive pulmonary disease: a randomised controlled trial. Lancet 353(9167): 1819-1823.

Vidgren, M., Karkkainen, A., Karjalainen, P. (1998). Effect of powder inhaler design on the deposition in the respiratory tract. Int. J. Pharm.; 42: 211-216.

Villafranca, C., Borzone, G., Leiva, A. and Lisboa, C. (1998). Effect of inspiratory muscle training with an intermediate load on inspiratory power output in COPD. Eur Respir J 11(1): 28-33.

von Matius, E. (2000). The enviromental predictors of allergic diseases. J Allergy Clin Immunol 105:9-19.

Waldeck, B. (2002). [beta]-Adrenoceptor agonists and asthma--100 years of development. Eur J Pharmacol 445(1-2): 1-12.

Waldeck, B. (2002). [beta]-Adrenoceptor agonists and asthma--100years of development. Eur J Pharmacol 445(1-2), 1-12.

Warren, S. J. and Taylor, G. (1998). Effects of flow profiles on the deposition of radiolabelled BDP from a novel dry powder inhaler (DPI, Clickhaler®),a conventional metered dose inhaler (MDI) and MDI plus spacer. In: Dalby RN, Byron PR, Farr SY (eds). Respiratory Drug Delivery Vol. V1 USA: Interpharm. Press Inc. 453-455.

Watson, D. G. (2005). Pharmaceutical analysis: A textbook for pharmaceutical students and pharmaceutical chemists. Second ed. pp221-233. Elservie Churchill Livingstone

Weibel, E. R. (1963). Morphometry of the human lung. New York, Springer Verlag and Academic Press: 151.

Wen, A. S., Woo, M. S. and Keens, T. G. (1997). How many manoeuvres are required to measure maximal inspiratory pressure accurately. Chest 111: 802-807.

Wijkstra, P. J., Mark, T., W. V., Boezen, M., Altena, R., Postma, D. S. and Koeter, G. H. (1995). Peak inspiratory mouth pressure in healthy subjects and in patients with COPD. Chest 107(3): 652-656.

Wilson, S. H., Cooke, N. T., Edwards, R. H. and G., S. S. (1984). Predicted normal values for maximal respiratory pressures in caucasian adults and children. Thorax 39: 535-538.

Wohl, M. (1998). Developmental physiology of the respiratory system. In: Chernick V, Boat TF, Kendig EL, Editors. Kendig`s Disorders of the Respiratory Tract in Children,5th ed, Philadelphia: Saunders19-27.

Wong, A. G., O'Shaughnessy, A. D., Walker, C. M. and Sears, M. R. (1997). Effects of long-acting and short-acting beta-agonists on methacholine dose-response curves in asthmatics. Eur Respir J 10(2): 330-336.

Wright, L., Brocklebank, D. and Ram, F. (2002). Inhaler devices for the treatment of asthma and chronic obstructive airways diseases (COPD). Qual Saf Health Care 11 (4): 376-382.

Zanen, P., Go, T. L. and J.W, J. L. (1996). Optimal particule size for B2 agonist and anticholinergic aerosols in patients with severe airflow obstruction. Thorax 51:977-980.